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ABSORPTION OF NUTRIENTS FROM SUBSOIL IN RELATION TO CROP YIELD

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(WITH NINE FIGURES)

Investigations of the development of root systems of native and crop plants have been pursued for a number of years at the University of Nebraska. The gradual intensification of this work has led to a type of experimentation designed to discover the nature of the activities of roots at great depths in relation to absorption of water and nutrients. In the present investigation the effects of absorption of nutrients from deep levels upon quantity and quality of yield were determined. Studies on the amounts of water and nitrates removed by crops from various soil levels were begun in 1919, but little attention was then given to yield. Since the results of these earlier experiments have a direct bearing upon the present problem, however, the methods employed being essentially the same, the more important results will be briefly summarized (cf. WEAVER, JEAN, and CRIST **20**).

Containers 1.5–3 feet in diameter and 2.5–5 feet deep were employed. They were placed in trenches, which were then refilled with soil, and crops planted around the containers in such a manner that the experimental crops in the containers were grown under field conditions. The containers were filled with well mixed soil of known water content and physical and chemical composition, to which, at certain levels, $NaNO_3$ had been added at the rate of

400 parts per million. The containers were filled in such a manner that the well compacted soil at any level occupied the same relative position as regards depth that it had occupied before removal from the field. The fertilized layers, and in some cases every 6-inch layer, were separated from the rest of the soil by wax seals which prevented the movement of water or solutes, but through which the roots readily penetrated. To prevent water intake, each container was furnished with an appropriate wooden roof. Crops of Manchuria barley, early Ohio potatoes, and maize were grown. In order to study the activities of the roots at various stages in their development, enough containers were used so that some could be examined at each of the several periods of growth. In fig. 1, containers 1 and 2 illustrate diagrammatically the development of the roots of barley when 55 days old and in the 6 or 7 leaf stage. In containers 3, 4, and 5 the crop was in blossom (74 days old), while in 6 and 7 it had reached maturity. The horizontal lines indicate the positions of the wax seals, and the double vertical lines the positions of the 6-inch levels to which the nutrient was added. The numbers at the bottoms of the containers give the nitrates in parts per million, based on the dry weight of the soil, absorbed from the fertilized layers. The gains by nitrification at the several levels were determined from the control container (no. 8), without a crop.

The amount of water absorbed by barley from the deeper soil (to 3.5 feet) was in direct relation to the growth of the root system into these deeper layers. The total amounts absorbed to depths of 2.5 feet in general were practically the same from the several 6-inch levels. Corn is an extravagant user of water, absorbing large quantities from the third and fourth foot of soil, and smaller amounts from the fifth foot. Potatoes absorbed water to depths of 2.5 feet. Potatoes used the nitrates in smaller amounts than barley. When beginning to blossom (74 days old) they had removed 143 and 70 parts per million of nitrates from the 1–1.5 and 1.5–2 foot layers respectively, and when beginning to ripen (100 days old) they had removed 228 parts per million at a depth of 1.5–2 feet, and 76 to 165 parts per million at the 2–2.5 foot level. Corn removed 203, 140, and 118 parts per million at depths of 3, 4, and 5 feet respectively.

From these experiments we may conclude that (1) the roots of crop plants are active in the absorption of both water and nitrate salts, even at the maximum depth of their penetration; (2) both water and nitrates are taken up from these lower portions of the soil in considerable quantities, although to a less extent than from

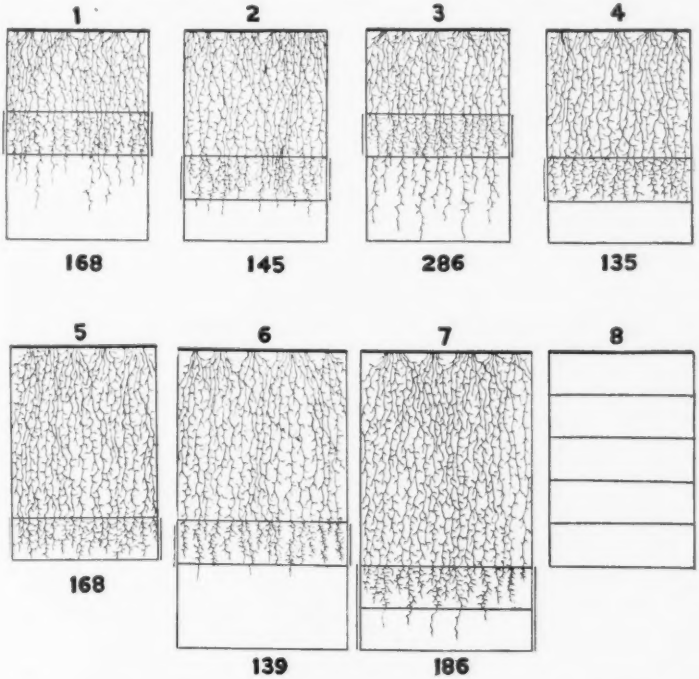

FIG. 1.—Diagrammatic representation of root development of barley at three different stages in growth; $NaNO_3$ occurred at several levels delimited by double vertical lines and horizontal lines, latter represent wax seals.

the more superficial portions; (3) the plants receive this supply of water and nitrogen from the deeper soil layers during the later and perhaps more critical stages of their development; (4) the roots branch more profusely and are more abundant where they come in contact with the fertilizer; and (5) there is urgent need for a series of experiments to ascertain the significance of absorption at great depths in relation to quantity and quality of crop yield.

An extensive literature elucidating the problem of fertilizers and their effects upon yield of the various economic crops has been produced. Practically none of it refers to the present type of investigation, because in all experimental tests and agricultural practices the application of fertilizer has been restricted to the surface or merely to the first four to eight inches of the soil. Among soil scientists it has long been the custom to take samples to depths as great as 4–6 feet and occasionally even deeper. While slight attention has usually been given to the composition of the deeper samples, yet it has meant a degree of recognition of the importance of the nature of the deeper soil and soil solution. Serious consideration of the lower subsoil has been taken only when it was of rock or contained a hardpan, or, in some other way than through a deficiency of nutrient substances became detrimental to crop production (**19**).

Experimental methods

Large, water tight oak barrels 22 inches in minimum diameter and 30 inches deep were used as containers. Sufficient soil to fill them was obtained from the first 2.5 feet of the soil in the field in which the experimental work was done. The soil was low in both nitrate nitrogen and active phosphoric acid (table I). While crops

TABLE I

NO_3 AND P_2O_5 CONTENT OF SOILS

Soil	Depth (ft.)	Nitrate (NO_3) (p.p.m.)	P_2O_5 (per cent)
Surface.....	0–1	99.5	0.029
Subsoil.....	1–2.5	11.1	0.012

on eastern Nebraska soils respond readily and consistently to nitrate fertilizers, experiments conducted by the department of agronomy of the University of Nebraska (unpublished data) have shown that phosphorus fertilizers have failed to stimulate production.

On March 27, 1922, two trenches were dug in the field where the crops were to be grown, and the containers, 18 in number, placed in them in rows with the tops about 4 inches above the general soil level. The soil had previously been brought to an approximately uniform water content of about 27 per cent. As the several

containers were filled, samples were taken at each level for moisture determinations, and also, where necessary, for the determination of nitrates. The fertilized layers were separated from the soil, both above and below, by means of wax seals, which consisted of 85 per cent paraffin and 15 per cent petrolatum. The seal was applied hot, so that it penetrated a little into the soil, and when it cooled clung tenaciously to the soil particles. It varied from 2 to 3 mm. in thickness. As shown in previous investigations, the seal

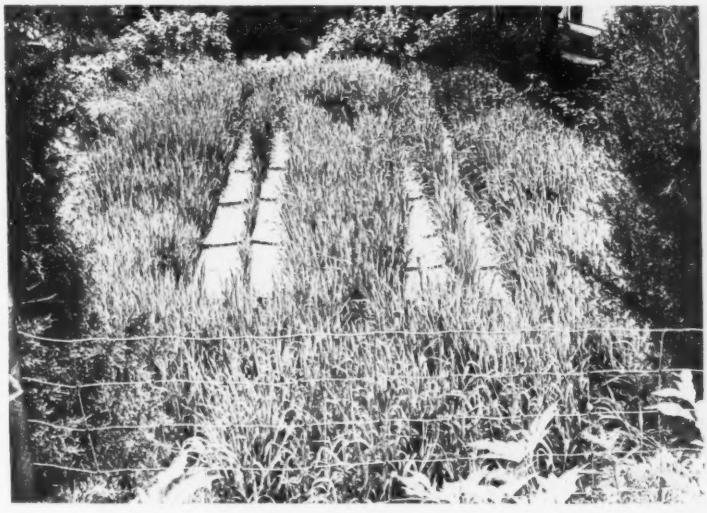

FIG. 2.—General view of experimental plat on May 29.

has no effect upon root development (**20**). The nutrient salts used were chemically pure monocalcium phosphate and sodium nitrate. The phosphate was applied at the rate of 1 gm. to 30 lb. of soil, which is approximately equivalent to 500 pounds of acid phosphate per acre. The nitrate was added at the rate of 292 parts per million of NO_3 on the basis of the dry weight of the soil.

When the containers had been filled to within 2 inches of the top, two thin wooden strips, about 1.5 inches in width and as long as the diameters of the containers, were placed edgewise and partially sunk into the soil so as to prevent the wax seal from cover-

ing the area of soil (about 1.5 inches in width) in which the crops were to be planted. Finally, the wax seal was covered with 2 inches of sand. Next a light green wooden roof, with sufficient slope to cause the water to run off, but with an opening 1.5 inches wide and as long as the diameter of the container, was fastened in place. A small amount of soil was placed in the openings thus left, and after the crop was planted about an inch of dry soil added to check evaporation (fig. 2). Seeds of Manchuria barley, selected

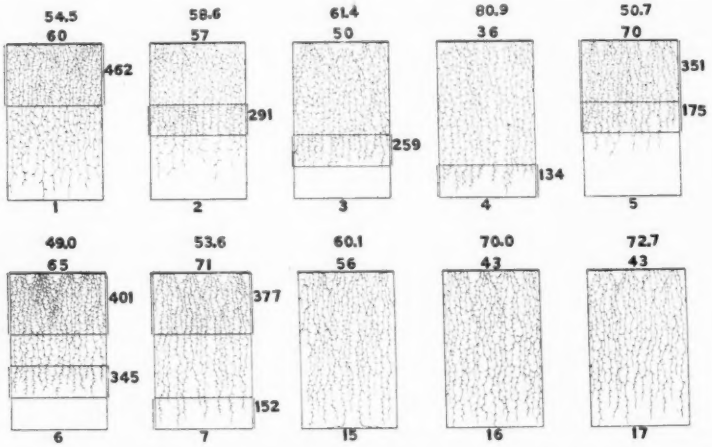

FIG. 3.—Diagrammatic representation of root development of barley fertilized with $NaNO_3$; seals and position of fertilizer indicated as in fig. 1; numbers immediately above containers represent number of plants, those higher, average length of stalks in centimeters.

for uniformity in size and previously treated with formalin solution to prevent smut infection, were sown on April 7, 100 seeds per container.

The position of the fertilized layers in the several containers in the nitrogen series, together with the unfertilized control crops, is shown in fig. 3, which also illustrates the relative root development at the time of harvest, June 26. The positions of the wax seals are indicated by horizontal lines and that of the fertilizer by double vertical lines. A control container without a crop or fertilizer, but sealed at every 6-inch level, was used as a check to determine the

extent of nitrification and denitrification. Root development, etc., at the time of harvest on June 24, is shown for the phosphorus series in fig. 4.

On May 2, when germination was completed and most of the seedlings had reached the second leaf stage, they were thinned to 35 per container. Eleven days later, just as tillering was beginning, enough of the smaller plants were removed to leave only 30 plants

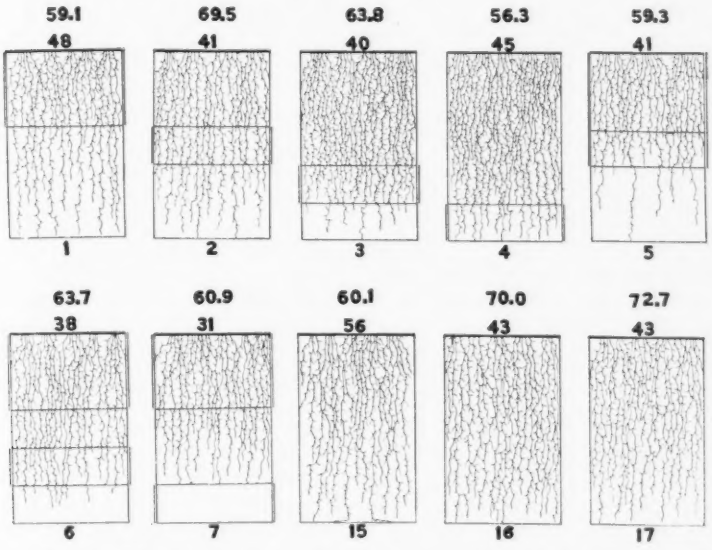

FIG. 4.—Diagrammatic representation of root development of barley fertilized with $CaH_4(PO_4)_2$; seals, position of fertilizer, number of plants, and length of stalks indicated as in fig. 3.

in each container, a number not in excess of that growing in a similar soil area under field conditions. The growth of the crops in the containers, as in previous experiments, paralleled that of the crop surrounding them, except for such differences as were caused by the fertilizer, which could readily be observed upon comparison with the controls. On May 22, and again on June 17, when very hot dry weather prevailed, the experimental crops were watered, the same amount of water (1 liter on the first date and 3 liters on the

second) being poured slowly into each container through the narrow openings through which the plants were growing. By June 1 the crop was heading. June 10–22 was a period of dry and unusually hot weather, which resulted in the general harvest of eastern Nebraska being at least 10 days earlier than usual. The experimental crops ripened very rapidly, and were harvested on June 24 and 26 respectively, the phosphorus series maturing first.

In harvesting the crop, the plants were cut off at the surface of the soil. Those from each container were wrapped separately in muslin cloths and taken to the laboratory, where they were spread out on tables in a well ventilated, well lighted room and allowed to dry. When thoroughly dry, the various measurements were taken, weights determined, and analyses made. In taking down the containers, they were tilted on their sides in the trench, the hoops chiseled off, the top staves removed, and the exposed core of the soil taken out in 6-inch sections in examining root development and obtaining samples for analyses. All soil samples for chemical analyses were taken in large composite form. Those to be analyzed for nitrates were treated with small amounts of toluol and sealed at once in mason jars. In the nitrate determinations the method of WHITING (**21**) was employed. Phosphorus determinations were made by digestion in fifth normal nitric acid for 5 hours at 40° C. This gave the quantity of the so-called active phosphoric acid (FRAPS **6**). The usual modified Gunning method was used in the analyses of grain and straw for nitrogen. Determinations of phosphorus in the grain and straw were made by digesting the samples with concentrated nitric acid in the presence of MgO, followed by precipitation from the extract with ammonium molybdate and the other steps in the gravimetric process.

Experimental results

NITRATE SERIES

The growth of root and shoot and the amounts of water and nitrates removed by the crop in the nitrate series, together with the water loss from the controls, are shown in table II. These data show the remarkable activity of roots in the absorption of both water and nitrates in the lower soil levels. Notwithstanding the

TABLE II

LOSS OF WATER AND NITRATES APRIL 7 TO JUNE 26

CONTAINER AND CROP DEVELOPMENT	DEPTH IN FEET	WATER CONTENT			NITRATE (NO_3) PARTS PER MILLION					
		April 7 (per cent)	June 26 (per cent)	Loss (per cent)	Original content April 7	Impregnation April 7	Gain by nitrification	Total	June 26	Loss
No. 1: 60 stalks, average length 54.5 cm., 42 heads; roots well developed in first foot, abundant to 1.5 feet, and fairly abundant to 2.5 feet	0–1	22.3	11.2	11.1	99.5	356.8	70.7	527	64.4	462.6
	1–1.5	22.4	16.9	5.5	.....	.....	.....	.....	.....	.....
	1.5–2	23.8	17.1	6.7	.....	.....	.....	.....	.....	.....
	2–2.5	23.3	18.3	5.0	.....	.....	.....	.....	.....	.....
No. 2: 57 stalks, average length 58.6 cm., 43 heads; roots well developed in first foot, very abundant at 1–1.5 feet, but sparse to 2 feet, a few extended to 2.1 feet	0–1	24.8	11.1	13.7	.....	.....	.....	.....	.....	.....
	1–1.5	22.6	16.8	5.8	11.1	362.9	24.9	398.9	107.5	291.4
	1.5–2	22.8	18.0	4.8	.....	.....	.....	.....	.....	.....
	2–2.5	25.2	25.0	0.2	.....	.....	.....	.....	.....	.....
No. 3: 50 stalks, average length 61.1 cm., 40 heads; normal development of roots to 1.5 feet; then very abundant and evenly distributed to 2 feet, below which practically none occurred	0–1	22.5	11.8	10.7	.....	.....	.....	.....	.....	.....
	1–1.5	25.3	18.0	7.3	.....	.....	.....	.....	.....	.....
	1.5–2	25.2	17.8	7.4	11.1	365.3	25.8	402.2	142.4	259.8
	2–2.5	23.2	23.2	0.0	.....	.....	.....	.....	.....	.....
No. 4: 36 stalks, average length 80.9 cm., 36 heads; roots abundant and evenly distributed to 2 feet, below which abundant but not uniformly distributed	0–1	23.4	12.2	11.2	.....	.....	.....	.....	.....	.....
	1–1.5	25.4	16.4	9.0	.....	.....	.....	.....	.....	.....
	1.5–2	23.7	17.9	5.8	.....	.....	.....	.....	.....	.....
	2–2.5	25.7	20.0	5.7	11.1	369.6	24.5	405.2	270.9	134.3
No. 5: 70 stalks, average length 50.7 cm., 47 heads; roots very abundant, profusely branched, uniformly distributed to 1.5 feet; few below this level and none beyond 1.8 feet	0–1	23.9	11.9	12.0	99.5	361.5	70.7	531.7	179.9	351.8
	1–1.5	24.3	17.4	6.9	11.1	361.8	24.9	397.8	222.3	175.5
	1.5–2	26.2	22.3	3.9	.....	.....	.....	.....	.....	.....
	2–2.5	23.4	22.9	0.5	.....	.....	.....	.....	.....	.....
No. 6: 65 stalks, average length 49 cm., 41 heads; roots well developed to 1.5 feet, fairly abundant and exceptionally well branched at 1.5–2 feet, none deeper	0–1	23.1	11.3	11.8	99.5	364.7	70.7	534.9	133.4	401.5
	1–1.5	25.0	16.6	8.4	.....	.....	.....	.....	.....	.....
	1.5–2	25.4	22.2	3.2	11.1	378.4	25.8	415.3	69.7	345.6
	2–2.5	22.9	22.5	0.4	.....	.....	.....	.....	.....	.....
No. 7: 71 stalks, average length 53.6 cm., 49 heads; roots very abundant, uniformly distributed to 1.5 feet, fairly abundant to 2 feet, not exceptionally abundant below 2 feet but exceedingly well branched	0–1	23.7	11.6	12.1	99.5	363.5	70.7	533.7	156.5	377.2
	1–1.5	24.6	16.5	8.1	.....	.....	.....	.....	.....	.....
	1.5–2	25.0	18.3	6.7	.....	.....	.....	.....	.....	.....
	2–2.5	29.8	24.9	4.9	11.1	378.7	24.5	414.3	261.9	152.4
Container without crop to determine nitrification and denitrification	0–1	25.0	23.9	1.1	26.6	.....	70.7	97.3	97.3	.....
	1–1.5	25.3	25.2	0.1	8.8	.....	24.9	33.7	33.7	.....
	1.5–2	25.7	24.4	1.3	22.1	.....	25.8	47.9	47.9	.....
	2–2.5	25.4	26.0	+0.6	8.8	.....	24.5	33.3	33.3	.....

TABLE II—*Continued*

Container and crop development	Depth in Feet	Water content			Nitrate (NO_3) parts per million					
		April 7 (per cent)	June 26 (per cent)	Loss (per cent)	Original content April 7	Impregnation April 7	Gain by nitrification	Total	June 26	Loss
No. 15:										
56 stalks, average length 60.1 cm., 41 heads; normal development of roots to 2 feet; fewer roots deeper but some reached 2.5 feet and ran along bottom of container	0–1	24.2	11.7	12.5			Unfertilized			
	1–1.5	24.9	16.8	8.1						
	1.5–2	24.8	17.8	7.0						
	2–2.5	27.4	21.7	5.7						
No. 16:										
43 stalks, average length 70.0 cm., 38 heads; roots very abundant in first 2 feet; fewer below, but extended to 2.5 feet	0–1	24.3	12.0	12.3			Unfertilized			
	1–1.5	23.9	16.9	7.0						
	1.5–2	23.9	16.4	7.5						
	2–2.5	27.6	21.0	6.6						
No. 17:										
43 stalks, average length 72.7 cm., 38 heads; roots very abundant to 2 feet, and fairly abundant to bottom of container	0–1	24.6	11.2	13.4			Unfertilized			
	1–1.5	22.6	14.7	7.9						
	1.5–2	23.8	17.8	6.0						
	2–2.5	27.3	23.2	4.1						

rapid ripening of the crop, due to abnormal weather conditions, water was absorbed in considerable quantities (3–13 per cent of dry weight of soil) at all depths to which the roots penetrated. The large amounts of nitrates removed at the several levels are indicated by numbers representing parts per million opposite these levels in fig. 3. When the fertilizer was placed both in the surface foot and at 1–1.5, 1.5–2, and 2–2.5 feet respectively, the amounts removed were, in order, 14.0, 61.5, and 14.5 per cent greater than when the fertilizer was placed in the surface foot alone.

The number of stalks developing from the 30 original plants in each container decreased progressively with the depth of the fertilizer from 60 to 36 (fig. 3). Except when the nitrates were below 2 feet, the number of stalks was greater than that of the average of the controls. In the doubly fertilized series the number varied from 65 to 71, which was 38–51 per cent greater than the average of the controls, and 8–18 per cent greater than when the fertilizer was placed in the surface foot only.

The average length of stalk increased progressively as the fertilizer was more deeply placed, ranging from 54.5 to 80.9 cm. (fig. 3). Except when the nitrates were below 2 feet in depth, the

stalks averaged shorter than those of the controls. In the doubly fertilized series the stalks were not only shorter than those of any of the controls, but also shorter than those of any of the singly fertilized series.

The number of heads was greater in every container (except no. 4) than the average of the controls. Where double fertilization obtained, the number exceeded that of the controls by 5–26 per cent. This statement may be misleading, however, unless the differences in the total number of stalks are taken into account. Column 7 of table III gives the percentage of stalks bearing heads, and shows that in every container (except no. 4, where the fertilizer

TABLE III

NUMBER AND LENGTH OF STALKS AND HEADS IN NITRATE SERIES

Container	Depth of fertilizer (feet)	Number of stalks	Average length of stalks (cm.)	Number of heads	Average length of heads (cm.)	Stalks bearing heads (per cent)
1	0.1	60	54.5	42	6.9	70
2	1–1.5	57	58.6	43	6.7	75
3	1.5–2	50	61.4	40	6.1	80
4	2–2.5	36	80.9	36	6.4	100
5	0–1 and 1–1.5	70	50.7	47	6.9	67
6	0–1 and 1.5–2	65	49.0	41	7.3	63
7	0–1 and 2–2.5	71	53.6	49	7.2	69
15	.....	56	60.1	41	6.7	73
16	.....	43	70.0	38	6.4	90
17	.....	43	72.7	38	6.4	90

was at 2–2.5 feet and was reached at a later period) a smaller percentage of stalks bore heads, and that the smallest percentages are associated with the doubly fertilized soils. The heads in the doubly fertilized series were longest and exceeded the average of controls by 6–12 per cent.

The average total dry weight of the controls (86.9 gm.) was exceeded by 12.2 per cent when the fertilizer was added to the surface foot only (table IV), but when the fertilizer was placed at lower levels, a decrease of 5.9, 15.5, and 4.9 per cent was determined in containers 2, 3, and 4 respectively. Adding nitrates to other levels in addition to the first foot resulted in an increase in dry weight. This gain amounted to 11.5, 11.3, and 26.9 per cent over the average of the controls in containers 5, 6, and 7 respectively.

The dry weight of the grain was decreased in every case where nitrate was applied either singly or doubly, except in container 7. This decrease was no greater in the doubly fertilized than in the singly fertilized series, nor is there any definite relation between the depth of the fertilizer and the depression in yield of grain.

The dry weight of straw was increased in container 1 of the singly fertilized series 24.9 per cent over the average of the controls, but where the nitrate was placed deeper than the first foot, a decrease occurred. The dry weight was equal to that of each of two of the controls, however, where the salt was at 1–1.5 and at 2–2.5 feet. The increases in the doubly fertilized soils were very

TABLE IV

DRY WEIGHT AND NITROGEN CONTENT OF GRAIN AND STRAW

Container	Depth of fertilizer (feet)	Dry weight of straw (gm.)	Dry weight of grain (gm.)	Total dry weight of tops (gm.)	Ratio of grain to straw	Nitrogen content of grain (per cent)	Nitrogen content of straw (per cent)
1.....	0.1	67.1	30.4	97.5	0.45	3.19	2.01
2.....	1–1.5	52.9	28.9	81.8	0.55	3.30	1.42
3.....	1.5–2	46.3	27.1	73.4	0.59	2.52	0.99
4.....	2–2.5	51.1	31.5	82.6	0.62	2.53	0.82
5.....	0–1 and 1–1.5	68.7	26.2	96.9	0.41	3.39	1.92
6.....	0–1 and 1.5–2	68.7	28.0	96.7	0.41	3.41	1.82
7.....	0–1 and 2–2.5	75.6	34.7	110.3	0.46	3.38	2.02
15.....	None	58.7	33.4	92.1	0.57	2.79	0.86
16.....	None	51.2	31.6	82.8	0.62	2.47	0.66
17.....	None	51.3	34.7	86.0	0.68	2.33	0.55

marked in every instance, being 27.9, 27.9, and 40.8 per cent in containers 5, 6, and 7 respectively. Furthermore, the increase in the doubly fertilized series over that of the crop where the soil was fertilized only in the surface foot was 2.4–12.7 per cent.

The application of nitrogen fertilizer caused reduction of grain in proportion to straw. From the data in column 6, table IV, it may be seen that the ratio of grain to straw is lower than the average of the controls in any container in which the crop was fertilized. The ratio increased as the amount of nitrate absorbed decreased. Thus it rose from 0.45, when the nitrates were in the surface foot, progressively to 0.62, when the nitrates were at a depth of 2.5 feet. It was less in the doubly fertilized series (except in container 7) than when the nitrate was added to the surface foot only.

A comparison of the data on the nitrogen content of grain and straw given in table IV shows that (1) the average nitrogen content of the grain of the controls (2.53 per cent) was exceeded by that of the crop from the singly fertilized series by 26.1 and 30.4 per cent respectively, when the fertilizer was at depths of 0–1 and 1–1.5 feet; (2) in the doubly fertilized soils the greater nitrogen content of the grain over the average of the controls was 34, 34.8, and 33.6 per cent respectively, as the salt was progressively deeper. Moreover, only one of the controls exceeded in nitrogen content of grain any individual of either of the two fertilized series. The grain from container 15 analyzed higher than that from containers 3 and 4. The average nitrogen content of the straw of the controls (0.69 per cent) was exceeded in every case by that of the fertilized plants. Moreover, any one of the controls had a much lower nitrogen content of straw than any crop in the fertilized series, except where the nitrate was below two feet. The increases, in sequence from containers 1 to 4, were 191.3, 105.8, 43.5, and 18.8 per cent, and for the doubly fertilized series (5 to 7) 178.2, 163.7, and 192.7 per cent.

Phosphorus series

The experiment with phosphorus fertilizer was identical with that of the nitrogen series as regards number of containers, position of seals, impregnated soil layers, etc., and the same control crops were used for comparison. The growth of the tops and roots, together with the amounts of water removed at the several levels, is shown in table V. A comparison of this table with fig. 4 shows the root activities at all depths, water being absorbed in quantities similar to those removed by plants of the nitrate series.

Data on the number and length of stalks and heads in the several containers are given in table VI. The number of stalks and the length of heads were less than the average of the controls in every case where fertilizer was used, except in the first container. Moreover, with the exception of container 2, the stalks were shorter and the heads fewer, but the percentage of stalks bearing heads was nearly equal to or greater than that of the average of the controls. In the containers where the soil was fertilized at two levels the stalks averaged fewer, and the heads were fewer and shorter than when

phosphorus was placed in a single layer. With one exception, however, the percentage of stalks bearing heads was greater in the doubly fertilized series.

TABLE V

LOSS OF WATER FROM PHOSPHORUS SERIES AND CONTROLS APRIL 7 TO JUNE 24

CONTAINER AND CROP DEVELOPMENT	DEPTH IN FEET	WATER CONTENT			POSITION OF FERTILIZER (feet)
		April 7 (per cent)	June 24 (per cent)	Loss (per cent)	
No. 1: 48 stalks, average length 59.1 cm., 38 heads; roots as abundant as in other unfertilized first foot of soil; below 1 foot less abundant than usual but extending to 2.5 feet	0–1	21.9	10.9	11.0	0–1
	1–1.5	23.1	17.8	5.3	
	1.5–2	24.8	19.9	4.9	
	2–2.5	28.1	25.5	2.6	
No. 2: 41 stalks, average length 69.5 cm., 39 heads; roots fairly abundant in first 1.5 feet, below which much fewer but some extended to 2.5 feet	0–1	23.9	11.9	12.0	1–1.5
	1–1.5	25.3	20.4	4.9	
	1.5–2	23.5	21.1	2.4	
	2–2.5	25.0	23.2	1.8	
No. 3: 40 stalks, average length 63.8 cm., 33 heads; roots very abundant, well distributed to 1.5 feet; fairly abundant to 2 feet, but sparse 2–2.5 feet	0–1	27.0	11.6	15.4	1.5–2
	1–1.5	24.8	17.3	7.5	
	1.5–2	22.4	19.6	2.8	
	2–2.5	25.8	24.2	1.6	
No. 4: 45 stalks, average length 56.3 cm., 37 heads; roots very abundant in first foot and more abundant to 2 feet than in any container of series; fairly abundant at 2–2.5 feet	0–1	24.2	12.2	12.0	2–2.5
	1–1.5	23.0	13.6	9.4	
	1.5–2	23.3	18.0	5.3	
	2–2.5	28.3	23.6	4.7	
No. 5: 41 stalks, average length 59.3 cm., 35 heads; roots very abundant, evenly distributed to 1.5 feet; very few found deeper, single root penetrated to 2.5 feet	0–1	25.1	12.9	12 2	0–1 and 1–1.5
	1–1.5	24.1	20.0	4.1	
	1.5–2	23.3	21.1	2.2	
	2–2.5	25.2	24.1	1.1	
No. 6: 38 stalks, average length 63.7 cm., 33 heads; roots very abundant, uniformly distributed to 2 feet, scarce in 2–2.5 foot layer except in center where they penetrated to 2.4 feet	0–1	26.5	11.9	14.6	0–1 and 1.5–2
	1–1.5	29.0	19.0	10.0	
	1.5–2	24.1	16.0	8.1	
	2–2.5	25.6	24.3	1.3	
No. 7: 31 stalks, average length 60.9 cm., 28 heads; roots abundant, rather evenly distributed to 1.5 feet, slightly fewer to 2 feet, below which none found	0–1	24.4	11.8	12.6	0–1 and 2–2.5
	1–1.5	20.2	19.1	7.1	
	1.5–2	25.2	20.9	4.3	
	2–2.5	23.6	23.0	0.6	
No. 15: 56 stalks, average length 60.1 cm., 41 heads; normal development of roots to 2 feet; fewer deeper but some reached 2.5 feet and ran along bottom of container	0–1	24.2	11.7	12.5	None
	1–1.5	24.9	16.8	8.1	
	1.5–2	24.8	17.8	7.0	
	2–2.5	27.4	21.7	5.7	
No. 16: 43 stalks, average length 70.0 cm., 38 heads; roots very abundant in first 2 feet of soil; fewer below but extending to 2.5 feet	0–1	24.3	12.0	12.3	None
	1–1.5	23.9	16.9	7.0	
	1.5–2	23.9	16.4	7.5	
	2–2.5	27.6	21.0	6.6	
No. 17: 43 stalks, average length 72.7 cm., 38 heads; roots very abundant to 2 feet and fairly abundant to bottom of container	0–1	24.6	11.2	13 4	None
	1–1.5	22.6	14.7	7.9	
	1.5–2	23.8	17.8	6.0	
	2–2.5	27.3	23.2	4.1	

Table VII shows that the average total dry weight of the controls (87.0 gm.) exceeded that of any of the crops where the soil was fertilized at any depth with phosphorus. In the singly fertilized soils, in the order of the increasing depth of the fertilizer, the

decreases were 11.3, 18.1, 24.7, and 23.7 per cent respectively. Where doubly fertilized the depression in yield was much greater, being, in the above order, 43.0, 36.6, and 51.6 per cent. These great decreases in dry weight occurred both in grain and straw. The depression of grain yield was somewhat greater than that of

TABLE VI

NUMBER AND LENGTH OF STALKS AND HEADS IN PHOSPHORUS SERIES

Container	Depth of fertilizer (feet)	Number of stalks	Average length of stalks (cm.)	Number of heads	Average length of heads (cm.)	Stalks bearing heads (per cent)
1.....	0–1	48	59.1	38	6.5	79.1
2.....	1–1.5	41	69.5	39	5.5	95.1
3.....	1.5–2	40	63.8	33	6.4	82.5
4.....	2–2.5	45	56.3	37	6.0	82.2
5.....	0–1 and 1–1.5	41	59.3	35	4.6	85.5
6.....	0–1 and 1.5–2	38	63.7	33	5.1	86.8
7.....	0–1 and 2–2.5	31	60.9	28	5.2	90.3
15.....	Control	56	60.1	41	6.7	73.2
16.....	Control	43	70.0	38	6.4	88.3
17.....	Control	43	72.7	38	6.4	88.3

TABLE VII

DRY WEIGHT AND PHOSPHORUS CONTENT OF GRAIN AND STRAW

Container	Depth of fertilizer (feet)	Dry weight of straw (gm.)	Dry weight of grain (gm.)	Total dry weight of tops (gm.)	Ratio of grain to straw	Phosphorus content of grain (per cent)	Phosphorus content of straw (per cent)
1.....	0.1	48.9	28.2	77.1	0.58	1.337	0.444
2.....	1–1.5	43.9	27.3	71.2	0.62	1.245	0.476
3.....	1.5–2	40.8	24.7	65.5	0.61	1.248	0.355
4.....	2–2.5	40.5	25.9	66.4	0.64	1.176	0.418
5.....	0–1 and 1–1.5	28.8	20.8	49.6	0.72	1.237	0.457
6.....	0–1 and 1.5–2	33.9	21.2	55.1	0.63	1.237	0.706
7.....	0–1 and 2–2.5	22.5	19.6	42.1	0.87	1.250	0.533
15.....	None	58.7	33.4	92.1	0.57	1.210	0.287
16.....	None	51.2	31.6	82.9	0.62	1.198	0.270
17.....	None	51.3	34.7	86.1	0.68	1.179	0.347

the straw when only one layer of soil was fertilized, but when a deeper layer in addition to the surface foot was impregnated, the depression of the straw yield was greater.

The ratio of grain to straw averaged 0.62 for the controls. In the singly fertilized series variations from this ratio were negligible, but in the doubly fertilized soils the ratio was considerably increased.

Lack of consistent correlation of the ratios with the phosphorus content of either grain or straw, however, indicates that they probably have little significance. The average phosphorus content of grain of the controls (1.196 per cent) was less than that from any container where the soil was singly fertilized above 2 feet. The increases, in the sequence of increasing depth of fertilizer, were 11.8, 4.1, and 4.3 per cent. Where the phosphorus salt was below 2 feet a decrease of 1.7 per cent was determined. In the doubly fertilized soils the gains, in the above sequence, were 3.4, 3.4, and 4.5 per cent. Furthermore, with the single exception just noted, none of the three controls equaled in phosphorus content of grain that of any of the fertilized crops.

The increase of phosphorus content of straw over that of the controls, which averaged 0.301 per cent, was greater than that of the grain. Moreover, each control had a lower phosphorus content of straw than that of any crop regardless of the depth at which the fertilizer was present. In the singly fertilized series, in order of increasing depth of phosphorus salts, the percentages were 47.5, 58.1, 17.9, and 38.9 respectively. Using the same sequence in the doubly fertilized series, they were 51.8, 134.5, and 77.1 per cent.

Discussion

The value of the data obtained from these experiments is enhanced by the fact that the crop was grown under field conditions and in containers sufficiently large to permit normal root development. Except for the effects brought about by the fertilizers, the crop developed normally. This was determined both by comparison with the controls and the crop in the field surrounding the containers. The effects of the various treatments of fertilizer became evident in the nitrogen series early in the development of the crop. Not only were the plants in contact with the nitrates more numerous, but also more bushy. They had longer and broader leaves, slightly thicker stems, and were a deeper green than either the controls or the plants fertilized with phosphorus. All of these characters were accentuated in the containers where nitrates occurred in the deeper soils as well as in the surface foot. Plants in contact with the phosphorus showed a decided glaucous appearance.

These differences in the two series, when the crop was 53 days old, are shown in figs. 5 and 6. The lateral spread of the tops of the plants on May 27 averaged 10 inches for the controls, 7.5 inches for the phosphorus series, and 13 inches for those fertilized with nitrates. The average greatest diameter of the second leaf from the top at this time was 14.4 mm. for the controls, and 15 mm. and 18 mm. respectively for the phosphorus and nitrate series. Head-

FIG. 5.—53-day old plants in container where soil was fertilized with nitrates at 0–1 and 1–1.5 feet.

ing occurred among the phosphorus fertilized plants on June 1, slightly in advance of the controls, and 5 to 7 days earlier than in the nitrate series. The condition of the crop at the time of harvest (June 24), where the nitrate and phosphate respectively occurred at 1–1.5 feet in depth, as well as that of a control, is shown in figs. 7–9.

The researches of NOBBE (**16**), THIEL (**18**), HÖVELER (**11**), FRANK (**5**), MÜLLER-THURGAU (**15**), VON SEELHORST (**17**), LIVINGSTON (**14**), BRENCHLEY and JACKSON (**1**), and many others are in

agreement that the presence of fertilizer increases root development. A greater development of the barley roots in the soil layers impregnated with the nitrate was found, but little or no difference was observed in the phosphorus series.

It is of interest that the roots absorbed nitrates at all levels into which they penetrated, notwithstanding the fact that the salt at all times was abundant in the surface soil. In fact, they extracted

FIG. 6.—Soil fertilized with phosphorus at 0–1 foot

almost as much from the surface foot in addition to the supply obtained from the deeper soil as when nitrate was supplied to the surface foot alone. This shows that the presence of salts in the subsoil profoundly affects the amount of nitrate absorbed, and this in turn materially affects the growth of the crop and the quantity and quality of the yield. The amount of phosphorus absorbed from the several levels was not determined, but judging from the variations in the phosphorus content of the plants, these statements seem to hold equally true for phosphate fertilizers.

Nitrates placed below but in conjunction with fertilizer in the surface foot failed to increase the nitrogen content of the straw beyond that of the crop fertilized in the surface foot only. In every instance, however, it resulted in an increased nitrogen content

FIG. 7.—Mature crops: soil fertilized with nitrates

of the grain. Conversely, under similar conditions when phosphorus fertilizer was employed, while the phosphorus content of the grain was not raised, that of the straw was increased in every case. Since phosphorus placed below as well as in the surface foot gave a better quality of straw, while under the same conditions

nitrogen caused a better quality of grain, it seems clear that when the limits of improvement in the quality of cereal crops through attention to the upper part of the soil have been reached, there are still possibilities of further progress through increased attention to the deeper strata.

FIG. 8.—Mature crops: with phosphates at 1–1.5 foot depth respectively

That nitrogen promotes tillering is well known. These experiments show that the addition of more nitrogen below the surface foot makes the tillering habit even more pronounced. GERICKE (7), in accounting for the abundant tillering and culm production in cultures of wheat which received additional supplies of nitrogen

late in the growing period, concluded that it was due to the greater extent of the root system. This resulted in the absorption of a larger amount of nutrients than was needed by the plants for normal development of the individual shoots. Our results are in agree-

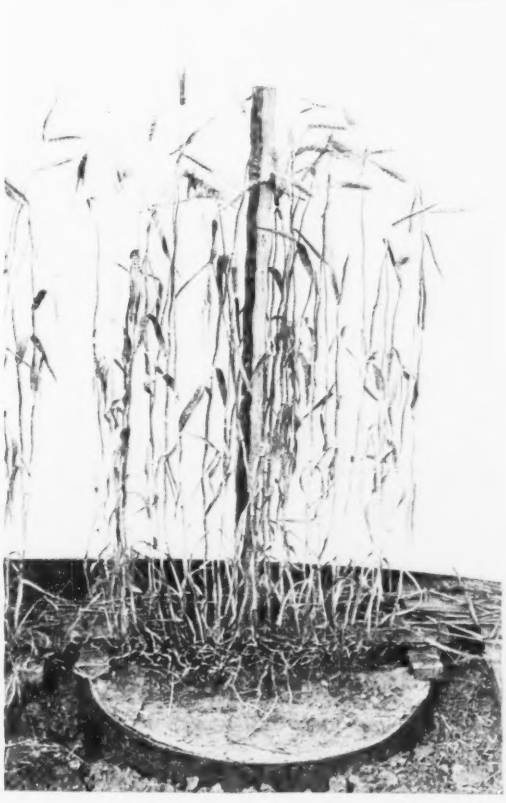

FIG. 9.—Mature crops: control, not fertilized

ment with this explanation. The number of stalks followed closely the amount of nitrate absorbed, and even more closely the nitrogen content of both grain and straw at maturity. Moreover, the amount of tillering correlated closely with the root area in contact with the nutrient, if we consider that the extent of the root systems

in the successive layers of the singly fertilized soils was progressively less from the surface foot downward, and greater in any soil doubly fertilized. The growth period of the plant, however, seems to affect the rate and significance of absorption more or less aside from the extent of root development.

Phosphorus depressed the yield. Larger quantities were absorbed where this salt occurred at deeper levels in addition to the surface soil, and the dry weight of tops was correspondingly depressed. The plants were fewer and smaller in almost every way. Reduction in yield varied almost directly in proportion to the amount of phosphorus absorbed. The average dry weight and average phosphorus content of straw of the controls, singly and doubly fertilized series, were respectively, 53.7, 43.5, and 28.4 gm., and 0.301, 0.423, and 0.565 per cent. In most cases, however, phosphorus increased the percentage of stalks bearing heads, while nitrates, except where they occurred below 2 feet, caused a decrease.

Nitrogen increased absolute yield only when present in the surface foot or in the surface foot as well as in lower levels. In either case the increase was brought about mainly through the straw and averaged greater under the second set of conditions. This effect through the straw was wrought simply by an enormous increase in the number of stalks. Nitrate depressed height growth, reduced the average weight per stalk, lowered the percentage of stalks bearing heads, and increased the total number of heads and their average length but little. It exerted only a slight influence upon the weight of the grain, yet the increase in the total number of stalks was sufficient to overcome these losses, and indeed to give increased totals. Unlike phosphorus, it lowered the ratio of the dry weight of grain to straw. This effect appeared where the plants absorbed more than 135 p.p.m. of NO_3, or where the straw contained above 1 per cent and the grain above 2.5 per cent of nitrogen. HELLRIEGEL and WILFARTH (**12**), working with sand cultures, obtained no further increase in the ratio of grain to dry matter of barley when the cultures were supplied with more than 168 mg. of nitrogen. HELLRIEGEL (**13**) also found that the percentage of grain to total dry weight of barley decreased when more than 113.6 mg. of P_2O_5 were supplied per pot.

The amount of nutrient removed from the soil is not the only factor affecting quality and quantity of yield. As regards nitrogen particularly, much seems to depend upon the time when the salts are available. Placing the fertilizer in the several containers in progressively deeper soil layers is really a means of varying the time in the life of the plant when the salt is available. And, since presumably roots absorb from deeper soil layers somewhat in proportion to the time they occupy these layers, it also varies the amount of fertilizer absorbed. Thus, as the time of nitrate absorption became progressively later in the life of the crop in the soil singly fertilized, so the number of stalks and heads, the nitrogen content of the straw, and, to a less degree, the nitrogen content of the grain decreased. Moreover, the same held true for the dry weight of both grain and straw and the average length of heads, where the fertilizer occurred above 2 feet. Where the nitrate occurred below 2 feet there was a relative increase in weight of grain and straw as well as length of heads, instead of a further decline. This occurred also in the doubly fertilized soils, where in addition gains were made in the nitrogen content of the straw, number and average length of stalks, number of heads, and percentage of stalks bearing heads. Thus applications of nitrate are most beneficial to the quantity and quality of the crop in the earliest and latest stages of root development and activity, although they are not entirely ineffective at certain intermediate stages.

The relation of the growth period of the plants to the effectiveness of the phosphorus was less marked, although very definite in some respects. As the contact of the roots with the fertilized layer was delayed, there was a progressive decrease in the number of stalks, dry weight of both grain and straw, and phosphorus content of grain.

GERICKE (**8**) applied sodium nitrate to spring and winter wheat, oats, and rye growing in soil in one gallon stone jars, at different growth periods. In the spring wheat and oats, the nitrogen or protein content increased continuously as the applications were made later and later in the life of the plant. Winter wheat and rye gave a like response only to applications made in the latest periods of growth. The barley in our experiment responded differ-

ently from these crops, in that the plants decreased in nitrogen content and in tillering as the availability of the nitrate was postponed through the mechanism provided in the singly fertilized series. GERICKE (**9**) confirmed his results regarding the relation he found between the time of application of nitrate salts and the resultant protein content of oat plants. He used a sandy soil deficient in nitrogen, and grew 7 plants in each one gallon container in a greenhouse. He found an increase in the number of stalks, but a decrease in their average height as the nitrate was applied later in the life of the plant. He believes the shorter stalks may be either a result of competition or due to the late production of tillers and their correspondingly shorter period for growth. Moreover, he found total dry weight and the weight of the grain to be greater when the nitrate was applied later in the life of the crop. In every respect these results are quite the converse of those set forth in this paper, and the reason for the difference seems to lie in the method of experimentation. It seems clear that cereal crops with roots which normally reach depths of 3–5 feet and spread laterally through a radius of 12 inches (WEAVER **19, 20**) might function quite differently when several plants were confined to 5.5 kg. of soil.

DAVIDSON and LE CLERC (**3**), working with wheat grown in outdoor plats, applied $NaNO_3$ at the surface at different periods of growth with the following results. Applications made early (plants 2 inches high) stimulated vegetative growth and consequently increased yield; applications made at the milk stage of the grain had no effect on either yield or quality; while applications at heading time gave a better quality of grain as to color and protein content, but the vegetative growth was unaffected. In 1922, DAVIDSON (**4**) again grew wheat in field plats of one square rod each and applied nitrates at different growth periods. The effectiveness of the nitrogen in increasing yield gradually decreased as the nitrate was applied later and later in the life of the plant. Its effectiveness, however, in increasing protein content of grain and percentage of grain to total dry weight of crop was increased. The data in table IV bear out these results except the effect upon the protein content of grain.

BURD (2) grew barley in containers with a surface area of 30×60 inches each and a depth of 18 inches. He concludes that the two elements (potassium and nitrogen) with which plant growth in general is most closely associated may approach or exceed their maxima at a comparatively early stage in the plant's development, that is, at the beginning of head formation; also that the mutual relations of soils and plants are such that it is generally desirable to have the large amounts of solutes incidental to relatively high concentrations in the soil solution at the commencement of the plant's growth cycle, but that it is unnecessary and may be undesirable to maintain this condition during the later stages of growth.

As regards nitrates, our results do not confirm this conclusion. WEAVER (20) has shown that the roots of barley when grown in a column of soil of adequate depth do not reach the lowest levels of their penetration to any considerable extent, and consequently do little absorbing there until the plants are nearing the blossoming stage. Root activities during the critical periods of blossoming and the filling of the grain have been shown to be exceedingly important. GILE and CARRERO (10), working with rice and corn where a part of the roots only were in contact with nitrogen or phosphorus in a complete solution, have shown that the smaller the portion of the roots in the complete solution the greater was the absorption of the elements per gram of roots.

Differences in the results obtained by various investigators on the use of nitrate fertilizers no doubt are due, at least in part, to the different crops and experimental methods employed. Moreover, it is possible that the effect of nitrates upon the development of a plant is determined in some measure by the portion of the root system in which the absorption occurs. Perhaps a smaller percentage of nitrate absorbed by the roots from the deeper soil levels actually reaches the above-ground parts, being used up in the metabolic processes of the roots themselves. If this is the case, the arrangement of the fertilized layers in the containers in this experiment would serve to make the results evident.

The fact that roots absorb nutrients at deep levels in the subsoil as well as from the surface layer should be given greater attention by all plant growers. The current idea that it is mainly the surface

layer of soil that supplies the plant with nutrients and that the subsoil is the crop's reservoir for water, should give way to the fact that it is the whole soil mass pervaded by roots that determines root activities. Since not only the quantity of nutrients but also the time at which they are absorbed affect quantity and quality of yield, the amount of available nutrients in the subsoil is a matter of great importance. In fact, the adaptability of a soil for crops may be determined largely by the composition of the subsoil. The problem of getting the phosphatic and potassic fertilizers, which do not leach extensively into the deeper soil where they may be more efficient, is one with which students of fertilizer practices should be concerned. The time and quantity of the applications of nitrates, which leach freely into the deeper soil, in relation to rainfall and root depth is a field worthy of investigation. It is possible that tillage and cultivation practices can be modified so as to give in advance of the crop season a supply of nitrates which might leach into the deeper subsoil in time for their absorption at the most effective period in the development of the crop.

Summary

1. This investigation was undertaken to determine the effects of absorption of nitrates and phosphates from the subsoil on quantity and quality of yield of barley. Eighteen containers, 22 inches in diameter and 30 inches deep, were filled with soil and subsoil and placed in trenches in the field. Thirty barley plants were grown in each container; they were surrounded by a crop of barley, growing partly in the refilled trenches. The soil, except in the controls, was fertilized at various levels to 30 inches in depth, and in some cases at two levels, with either $NaNO_3$ or $CaH_4(PO_4)_2$. Wax seals, through which the roots easily penetrated, were used to prevent the movement of water and nutrient salts from the fertilized layer to the soil above or below.

2. The roots in the controls reached a depth of 30 inches, but nitrate fertilizer at any level tended to lessen root depth and greatly increased branching. Phosphates did not noticeably increase root development.

3. Nutrients were absorbed in large quantities at every level to 30 inches. Although the plants used the largest amount of salts

from the surface foot, they also took large additional quantities from the deeper levels when it was available.

4. Absorption of nutrients at levels below the surface foot affects materially the quantity and quality of the yield. It does not lose its additive effect even when the surface foot is abundantly supplied with a similar nutrient. Thus the chemical composition of the subsoil and the soil solution is very important.

5. Nitrates increased total dry weight when applied to the surface foot early in the life of the plant. This results from its effectiveness in promoting heavier tillering. It increased dry weight and also quality of grain still more when available at lower levels as well as in the surface foot. Phosphorus depressed yield, particularly that of the straw, somewhat in proportion to the amount absorbed.

6. Time of absorption is an important factor. The effects of the nutritive salts are most marked on both quantity and quality of yield early and late in the development of the plant, that is, when absorption is confined largely to the first foot of soil and the crop is tillering, and again when the younger portions of the longer roots are absorbing from the deeper levels at the time of heading. Thus an ample distribution of the deeper portion of the root system in a rich subsoil solution at the later critical period of growth is exceedingly important. Consequently, a knowledge of the development and extent of the roots of crop plants is of primary interest.

7. These experiments show the importance of the subsoil as a source of nutrients for crops, and the effects upon plant development. They emphasize the values to be gained by fertilizer practices which take the composition of the subsoil into account.

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MEIOSIS IN POLLEN MOTHER CELLS OF OENOTHERA FRANCISCANA SULFUREA

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(WITH PLATES XIV, XV)

Introduction

Oenothera franciscana sulfurea is an evening primrose with cream colored flowers, which arose in 1914 in the experimental gardens of B. M. DAVIS. An account of its appearance and subsequent history, for which I am indebted to Professor DAVIS, can be summarized briefly as follows:

1912. Plant 12.16*a* *biennis* was pollinated by *franciscana* B. (pollen supplied by H. H. BARTLETT, from his culture of *franciscana* in Washington).

1913. Culture 13.36, F_1 hybrids of *biennis* (12.16*a*) × *franciscana* B. This culture was fairly uniform (DAVIS **4**), and resembled *franciscana* in most characteristics.

1914. Culture 14.56, from plant 13.36*a*, selfed. Germination was very poor (8 per cent), and the culture was small. The 51 plants which sent up flowering shoots were uniform and resembled closely the F_1 parent, 13.36*a*, except for one plant (14.56*w*), which differed from the rest in having cream colored ("white") flowers (DAVIS **5**). This was the first *franciscana sulfurea*, and its history in selfed line has been as follows:

1916. Culture 16.23 from plant 14.56*w*. Contents of one capsule, forced to complete germination, gave 188 seedlings and 32 empties. Germination 85 per cent; 64 plants were grown to maturity, all white flowered.

1917. Culture 17.23, from plant 16.23*a*. Earth-sown; 12 plants were brought to maturity, all white flowered.

1920. Culture 20.23, from plant 17.23, I 3. Earth-sown; 25 plants were grown to maturity, all white flowered.

1921. Culture 21.23, from plant 20.23–6. Contents of one capsule forced to complete germination gave 354 seedlings and 49

empties. Germination 87.8 per cent; 23 plants were grown to maturity, all white flowered.

There is reason to believe that the *sulfurea* character has been inherited from the *biennis* side. *O. franciscana* has been grown now for eight generations, and has yet to show any sign of aberrancy. On the other hand, in *biennis*, as is well known, a small percentage of aberrant forms is to be expected, among which are a few *sulfurea* forms. STOMPS (7) found 4 *sulfurea* plants in 920 plants of *biennis* (0.413 per cent), and DE VRIES (8) found 27 in 8500 (0.317 per cent). The *sulfurea* character, therefore, may easily have come from the *biennis* parent. In most other visible characters the plant resembles *O. franciscana*, and for this reason it has been named *O. franciscana sulfurea*. It will be observed that, so far as the *sulfurea* character is concerned, this strain up to the present time has bred entirely true. Cultures have not been grown of sufficient size as yet, however, to warrant any statement regarding the genetical characteristics of the plant as a whole, beyond the fact that they have exhibited a certain amount of variation.

Material and methods

Material was collected during the summer of 1920 in the experimental gardens of Professor DAVIS at Ann Arbor, Michigan. Several killing fluids were used, but, as in the case of *O. franciscana*, it was found that by far the best results were obtained by fixing for three or four hours in one of ALLEN'S modifications of Bouin's solution, containing picric acid (saturated aqueous solution) 75 cc., formalin (commercial) 25 cc., glacial acetic acid 5 cc., chromic acid 1.5 gm., urea 2 gm. The material was imbedded in paraffin, cut at 8–10μ, and stained with iron-alum haematoxylin.

Description

Longitudinal sections of the anthers of *O. franciscana sulfurea* resemble very closely those of *O. franciscana* at corresponding stages. Archesporial and pollen mother cells of the two forms are alike as to size and arrangement, and their nuclei are also of the same size. The separation and rounding off of the pollen mother cells take place at the same time and in like manner. The resemblance

is so close that, except for intra-nuclear differences at certain stages, sections from the two plants are practically indistinguishable.

Archesporium

In the archesporial stage the resemblance extends even to the nuclear contents. There is the same delicate and copious reticulum seen as in *O. franciscana* (Cleland 1), irregularly roughened and thickened with chromatin aggregations of varying size and shape, and in intimate contact with the nucleoli, of which one is usually much larger than the rest, if more than one is present. There is also the same absence of parallelism, the threads showing little if any tendency to pair. This condition is not only true of the archesporial resting stage, but of later stages as well. As the nucleus begins to approach the heterotypic prophase, some of the threads seem to shorten and thicken, which results in the stretching and attenuation of others. As a consequence, the meshes of the network become more irregular in size, and occasionally threads are brought into parallel relation with one another, a condition which might be interpreted as the parallelism of homologous threads. Such a large proportion of the threads remain undoubtedly single, however, and the occasional parallelisms are so obviously a result of the contractions and irregular amalgamation of threads which finally lead to the formation of the "open spireme," that there does not seem to be any reason for believing that a fusion takes place at this stage, either of homologous parts of two different spiremes, or of the split halves of the same spireme.

Heterotypic prophase

Synizesis.—Early and mid-synizesis stages resemble exactly corresponding stages in *O. franciscana*. The reticulum contracts in the same way to one side of the nucleus. This is usually the side which is nearest the periphery of the cell, which suggests the possibility that synizesis is an artifact, due to the influence of the fixing fluid. The nucleolus also leaves its original position in the interior and moves to the same side of the nucleus, where, buried in the reticulum, it becomes plastered up against the periphery as a somewhat flattened, cushion-shaped, black staining body. The

contracted mass of threads becomes so compact that it is impossible to see the individual threads, except along the periphery. Thin tangential sections and a study of the lateral surfaces of the knot are necessary in determining the situation at this stage. Most of the threads have become exceedingly tortuous and much wrinkled, as though they had shrunken. They are very unequal in diameter, some seeming to be increasing in importance and others diminishing, as described for *O. franciscana*. The spaces between the threads in such a contracted knot are very small of course, but there is no indication that threads parallel one another for any distance. There is rather a dense tangle of threads, which run in all directions, seemingly without order or system.

As synizesis proceeds, the number of threads decreases by the transfer of material composing certain threads into others, and by the fusion, in various ways, of adjacent parts of the reticulum. The surviving threads become more prominent, and the meshes in the network become larger, even though the synizetic knot as a whole does not increase in size. Little by little these threads begin to be distinguishable in the knot when examined as a whole, at first rough and unequal in diameter, but later becoming smoother and more uniform. As the threads become more prominent, the nucleoli flatten out more and more, the large one often assuming the form of a horseshoe or ring, or even fragmenting.

Thus far, the development of the nucleus has been so like that of *O. franciscana* that figures illustrating the latter will do equally well for this plant (CLELAND 1, figs. 1–8). The manner of the unfolding of the knot, however, differs in detail in the two plants. In *O. franciscana* it was found that the threads nearest the nucleolus became gorged and swollen with chromatin, which passed out from the latter, and gradually diffused throughout the reticulum. The thread system, with its chromatin content thus augmented, unfolded and became a diffuse and open reticulate spireme, to a large extent filling the nucleus, a typical "open spireme." In *O. franciscana sulfurea* the chromatin contents of the nucleolus pass into the reticulum also, but in a slightly different way. The flow seems for the most part to be confined to one or two threads which lead directly from the nucleolus to the center of the knot (figs. 1–3).

These become gorged and greatly swollen. Chromatin material passes along these to the center of the reticulum, and from there into the other threads. At the same time, the synizetic knot begins to unfold slowly. The main part of the spireme, composed by this time of threads which for the most part are prominent enough to be visible, leaves the region of the periphery and the nucleolus, and comes to lie in the center of the nucleus, leaving only a few strands bending and twisting in an open network between itself and the nucleolus. Through this openly reticulate portion pass the swollen threads, forming a broad, black staining, and straight band. There is no question in my mind that material is flowing through these threads into the reticulum. The attachment of the nucleolus to the broad band or bands is unmistakable, and a study of the nucleolus shows clearly that it is being emptied. At first it is uniformly black staining (figs. 1, 2). As the threads begin to swell and stain black, however, the edges of the nucleolus commence to lose their staining qualities. The black staining portion gradually becomes smaller and smaller, and more and more confined to the region around the exit (fig. 3). Soon the endonucleolus appears, freed from its covering of chromatin, a black staining little body in the almost unstained and empty looking portion of the nucleolus. The black color finally disappears entirely from the nucleolus and it is free from chromatin (fig. 4). Meanwhile the threads of the reticulum which are connected with the other end of the broad band have become swollen and black also, as the chromatin is passed out into the rest of the network. Gradually the chromatin diffuses through the reticulum, until at last it is distributed more or less evenly throughout the whole system.

Open spireme.—The open spireme (fig. 4) on the whole is not as open as it is in *O. franciscana*, nor is it diffused through as large a portion of the nucleus. Throughout the entire stage there is usually a region toward the center where the threads are crowded together into a rather dense tangle, and the looser portions of the reticulum as a rule are confined to a fairly small area. The threads fail to show any indication of a longitudinal split or of parallelism, even in those nuclei which have been very much destained. The presence of chromomeres can generally be demonstrated. Some-

times they are very prominent, strung along the spireme like beads on a string. At other times they are more or less crowded together, so that their individuality is partially or entirely obscured.

SECOND CONTRACTION.—As the second contraction period draws near, the central, more densely tangled region, which has never entirely disappeared, becomes more prominent, for all of the threads begin to contract, commencing at the center. At the same time, the peripheral portions of the reticulum become thrown into more or less clearly defined loops of varying size and appearance (figs. 5, 6). Contraction continues for some time, and, as the threads in the central region become more and more swollen and closely packed, they gradually form a roughly spherical aggregation, so dense that its structure cannot longer be determined (fig. 7). At the same time the peripheral loops shrink in size. In some cases this means a corresponding increase in the diameter of the threads making up the loops, but more often it seems that most of the material composing them flows or migrates into the more central portion of the reticulum, so that the loops themselves, although smaller, are composed of no thicker threads than they were before contraction began. Occasionally the loops become greatly narrowed, the two sides approaching one another until in some cases they become contiguous. As these loops radiate out from the central knot, it sometimes happens that a pair of them may lie so near to each other that at first sight one is tempted to consider them the separated halves of a larger loop, each half consisting of two parallel threads, a condition which would strongly suggest parasynapsis. A careful study of the way in which these structures are formed in the earlier stages of contraction, however, makes it clear that they are individual loops, the unsplit sides of which have approximated (fig. 6).

Contraction continues until in many cases the loops practically disappear into the central knot. In a considerable proportion of the cells, however, the loops do not entirely disappear. One can find nuclei in all stages of second contraction, in which the outer ends of the loops can be seen protruding beyond the edge of the knot (fig. 7). At first the component threads are thin. Contraction has not caused them to increase in diameter in many cases,

because their contents seem to have largely been removed toward the interior. After the period of maximum contraction has been reached, however, material begins to flow back again. The threads making up the loops increase in thickness, and the loops become more prominent as a result.

FINAL PROPHASE STAGES.—The compact second contraction knot begins to loosen as the nucleus passes into the stage generally known as diakinesis, and little by little resolves itself into a spireme, now greatly thickened, of which the loops which were usually observed during the contraction period constitute a part (figs. 8, 9). At first greatly tangled, especially toward the center, the spireme in a short time becomes more or less completely unfolded, revealing an arrangement of the chromosomes at the same time interesting and unusual (figs. 10–13). In the first place it will be observed that the spireme does not, as in most plants and animals, break up into pairs of homologous chromosomes which separate and stand apart during diakinesis, but remains intact and unbroken. In the second place, it can easily be seen that the chromosomes are arranged end to end in the spireme. They are somewhat spindle-shaped, thickest in the middle and tapering at the ends, and are usually attached only by a thin thread. Consequently they are clearly distinguishable and can be counted with ease, the diploid number always being found. There can be no question that at this period the spireme is univalent, consisting of only one row of chromosomes, which are attached end to end, or telosynaptically.

The appearance at this time is interesting for a third reason. The chromosomes are always arranged in such a way that the spireme is formed into a definite and characteristic figure, which is present in all nuclei at this stage. It will be remembered that in *O. franciscana* also the chromosomes are joined together in a very definite and constant manner in diakinesis. In the latter plant four of them are attached end to end to form a closed circle, to which are linked three smaller rings, each consisting of two chromosomes; and to two of these there is linked in turn another ring, made up also of two chromosomes. In *O. franciscana sulfurea* the chromosome arrangement is as definite and constant as it is in *O. franciscana*, but it is different, being somewhat simpler, and in some ways a

more striking grouping. Of the fourteen chromosomes, twelve are joined end to end to form a large closed circle, and to this circle is linked one small ring, composed of two homologous chromosomes. The circle of twelve is usually too large to be accommodated in the nucleus without a certain amount of twisting and turning, but there is not the slightest difficulty in most cases in following along the chain from a given point back to the same point again, and in determining that the number of chromosomes making up the circle is twelve. The individual chromosomes at this time are rather large, and usually stand apart distinctly. Their surfaces are uneven and soft looking, and one gets the impression that probably they are quite spongy in texture.

It is generally necessary to have the whole nucleus in order to see the ring in an unbroken and complete condition, and when sections are cut at 8–10 μ there will not be a very large proportion of entire nuclei. Practically every whole nucleus examined at this stage, however, has shown the unbroken circle clearly, and thirty-seven very clear cases have been recorded. In addition, a large number of incomplete nuclei show from eight to ten chromosomes in a string, with the separate ring often appearing, so there can be no doubt that a circle of twelve chromosomes, plus another pair, constitute the normal arrangement at this time.

The end to end arrangement of so many chromosomes confirms very strikingly the conclusion that telosynapsis is present here. Were there but six or seven chromosomes at the most so attached, we might conclude that in the earlier prophase stages the chromosomes had formed a bivalent spireme, the halves of which had subsequently separated. When we find twelve of the fourteen chromosomes placed end to end, however, it can hardly be doubted that the spireme has been univalent from its beginning, and has failed to segment transversely into its bivalent components.

The constantly uniform placing of the chromosomes also tends to emphasize the suggestion previously made (1) that the chromosomes may be distributed in earlier stages of the heterotypic prophase, and perhaps even in somatic nuclei, according to a definite system, and not merely in a chance manner. We now have two plants which just previous to the heterotypic metaphase show an

arrangement of chromosomes characteristic and uniform for the form, which may point toward a more perfect orderliness and greater uniformity in nuclear structure than has hitherto been realized.

The nucleolus behaves during this period exactly as did that of *O. franciscana*. It is found plastered up against the nuclear membrane, where it gradually melts away and disappears. The little endonucleolus is also present. This arrangement of chromosomes persists throughout the entire period. The twelve chromosomes making up the large ring remain attached. The small ring, however, may separate from the large one and lie independently in the nucleus (figs. 11, 12). During the latter part of this stage, spindle fibers begin to appear, closely investing the nuclear membrane, and when the latter melts away, they penetrate rapidly into the region of the nucleus. Meanwhile the chromosomes shrink greatly, until they are scarcely one-fourth the size previously presented (figs. 14, 15).

Heterotypic metaphase

At first the spindle is multipolar, but soon it becomes bipolar. Enmeshed in the maze of spindle fibers are the chromosomes, still attached to one another, and lying in an irregularly tortuous cluster (fig. 15). The bivalent pair, if it has not already separated from the circle of twelve, does so some time during this period.

In view of the fact that the chromosomes are still joined together, and the bivalent pairs do not act separately as independent units, we should not expect to find in this plant that regularity of chromosome position in metaphase, and separation in anaphase, so characteristic of *O. franciscana*, in common with most plants and animals. It is surprising, therefore, to what degree normal distribution of the chromosomes to the poles is found in spite of what we should suppose would be handicaps to such behavior. The chromosome chain lies irregularly contorted and twisted in the midst of the spindle fibers. We should expect the chromosomes to become attached to whatever fibers approach them most nearly, irrespective of from what pole these might emanate. Even if the chain were not so twisted, but lay horizontally across the cell, so that the chromosomes were all equidistant from the two poles, it is not easy to see

what would prevent such a chance attachment. When chromosomes, instead of all being joined together, are paired in diakinesis, as they are in most organisms, it is natural that the members of a pair should become attached to the fibers leading to different poles, for in practically every case one chromosome lies nearer one pole, and the other nearer the other pole. There is every reason to suppose, however, that it makes no difference which chromosome of a pair is nearer a given pole; the position of the two chromosomes of a pair, with reference to the poles, is regulated entirely by chance. If, then, in the cells of typical plants, chance determines which chromosomes of a bivalent pair shall become attached to fibers going to a certain pole, there is little reason to believe that the situation in *O. franciscana sulfurea* is different. In this plant also it seems to be entirely a matter of chance which pole a given chromosome is nearest, as it lies attached to its neighbors in the winding twisted chain, and we should expect, therefore, an entirely unregulated attachment to the fibers, and distribution to the poles.

It is surprising to find, therefore, that this process takes place in a markedly regular manner. In most nuclei the chromosomes in the circle of twelve alternate in their attachment (figs. 16, 18). Fibers from one pole will become attached to the first, third, fifth, etc., chromosome of the chain, and those from the other pole to the second, fourth, sixth, etc. They are usually fastened to the middle of the chromosome. When the fibers begin to contract, the equal pull from the two poles brings the circle of twelve into a horizontal position across the cell. The chromosomes, still attached to one another at their ends, but pulled in opposite directions by the fibers fastened to their mid-regions, become *V*-shaped, so that the chain as a whole takes on a regular zigzag appearance. Meanwhile the independent bivalent pair of chromosomes acts in a perfectly normal manner.

The chromosomes continue their attachment throughout metaphase. The spindle fibers appear to exert a pull upon the chromosomes, and they are stretched farther and farther apart (fig. 18). Their ends grow more and more attenuated, until at last they can stand the strain no longer, and snap. The chromosomes are thus set free and quickly pass to the poles. It is not

until anaphase, therefore, that the circle of twelve chromosomes is finally broken up.

While in general the chromosomes are distributed equally and regularly to the poles, in some cases the distribution is unequal (figs. 17, 20). Occasionally a chromosome will become attached to fibers from the wrong pole, so that two adjacent chromosomes are pulled in the same direction. This results in eight chromosomes going to one pole and six to the other. I have tried to estimate the proportion of cases in which the chromosomes are thus unequally distributed, by studying the cells during late interkinesis (figs. 22, 23). At this stage the chromosomes stand apart clearly, and it is possible to tell accurately in a large majority of cells whether seven went into each daughter nucleus, or some other number. At this stage 623 cells were complete enough to be certain of the chromosome number. Of these, no cell was found in which the number going to one pole exceeded eight. On the various slides studied, the proportion of cells in which the chromosomes had been irregularly distributed, eight going to one pole and six to the other, ranged from 11 to 30 per cent. The average for all the cells counted was 16 per cent. This probably represents fairly accurately the average percentage of irregular distribution in this plant, a strikingly small percentage when one considers the way in which the chromosomes are united during metaphase.

Anaphase to interkinesis

Once separated, the chromosomes pass rapidly to the poles. In most cases they are in the form of short, fat *V*'s, but as yet show no signs of a longitudinal split (fig. 19). At the poles they are grouped closely together (fig. 21), and are soon inclosed by a nuclear membrane. A central chromosome is usually found, surrounded by six peripheral ones, although this arrangement is upset when six or eight are present instead of seven. From now on the pollen mother cells are developed, and the pollen grains are formed, much as in *O. franciscana*. As the daughter nuclei grow, the chromosomes form slight attachments to one another. They become irregular in outline, but their identity is rarely lost. Occasionally there are signs in early interkinesis of the split in the chromosomes prepara-

tory to the homoeotypic division, but as a rule this does not appear until later, when the chromosomes have lost all contact with one another. The fact that the split has taken place finally becomes evident when the two grand-daughter chromosomes separate along most of their length, and remaining in contact only at the middle, twist around in such a way as to form a maltese cross (figs. 22, 23). Nucleoli are developed *de novo* in contact with the chromosomes, but do not reach a large size. In all respects, therefore, interkinesis in this plant is like that in *O. franciscana*.

HOMOEOTYPIC MITOSIS

No wall is formed between the daughter nuclei. As the time for the homoeotypic metaphase approaches they begin to shrink, and spindle fibers make their appearance. The two nuclear membranes disappear simultaneously, and the chromosomes are enmeshed in multipolar spindles, which soon become bipolar. By this time the chromosomes have lost their *X* shape, the two halves of each having condensed into a pair of small, almost spherical bodies, which lie more or less closely appressed (fig. 24). The homoeotypic metaphase differs markedly from the heterotypic because of the rounded rather than *V* shape of the chromosomes, and by the fact that these are in pairs, which are not connected with one another. The two metaphase figures lie in opposite sides of the cell, sometimes in one plane, but often at right angles to each other, or at least in different planes (figs. 25, 26).

The grand-daughter nuclei are constituted as were the daughter nuclei, the six, seven, or eight chromosomes being very close together at first, but separating as the nucleus grows. The nucleus passes rapidly into a resting condition, the process being similar to that already described for *O. franciscana*. The chromosomes do not split, but send out delicate strands which meet and fuse, forming an open network upon which is distributed in irregular fashion some of the chromatin. When the resting condition is finally attained, the chromosomes have so amalgamated that their individuality is completely lost (CLELAND 1, figs. 36–38).

The four nuclei lie equidistant from one another in the cell. The more or less prominent sets of spindle fibers developing between

them in *O. franciscana* have not been noticed in this plant. The walls separating the spores are not developed until the nuclei are completely at rest; then they are formed after the manner described for *O. franciscana*. All of the walls are formed at the same time. The first trace of their appearance consists in delicate cleavage lines, appearing at the periphery of the protoplast midway between the nuclei. These run quickly into the interior, meet, and soon grow into much wider walls. The process is probably very rapid, as early stages seem to be rare. The wall thickens much more rapidly toward the outside than farther in, so that the protoplasts are separated more widely at the exterior. No cell plates have been found, and it seems quite certain that the walls are formed by very rapid furrowing, which proceeds from without inward.

Discussion

In some respects the cytological development of the pollen grains of *O. franciscana sulfurea* bears a strong resemblance to that in *O. franciscana* (CLELAND 1), to which it is closely related, emphasizing the suggestions and confirming the conclusions drawn from a study of the latter. In certain particulars, however, the two plants show differences in development which are of interest and importance.

CYTOLOGICAL

From the cytological standpoint, the situation in *O. franciscana* was of interest principally for two reasons: (1) it presented a clear case of telosynapsis; (2) the chromosomes in diakinesis showed a constant and definite arrangement, which was both striking in appearance and suggestive of a higher degree of order in the nucleus than has perhaps generally been imagined. Both of these points are clearly emphasized in *O. franciscana sulfurea* also.

TELOSYNAPSIS.—If anything, *O. franciscana sulfurea* shows telosynapsis even more clearly than does *O. franciscana*. In early heterotypic stages, both plants are alike in displaying very little that could be used as evidence for the synaptic pairing of two univalent spiremes to form a bivalent one, or for that matter, for the presence of a longitudinal split in a univalent spireme. This is true of pre-synizetic stages as well as of all subsequent ones. In

addition to negative evidence of this sort, there is positive proof that chromosomes are placed end to end in a univalent thread system. The unsplit spireme of the "open spireme" stage forms itself into loops. These loops can be observed in all stages of second contraction, and when the unfolding process occurs, it can be seen that from them the chromosomes have been formed without any splitting having occurred. The chromosomes are but segments of the whole spireme, therefore, and must have been placed end to end during the spireme stages.

O. franciscana sulfurea also furnishes convincing evidence of the presence of telosynapsis in the way the chromosomes are arranged after this unfolding takes place. At this stage, twelve of the fourteen chromosomes are found to be attached end to end in all nuclei, and form a large closed circle. Such a condition makes it difficult to accept any interpretation other than the telosynaptic one.

CHROMOSOME ARRANGEMENT.—An arrangement of the chromosomes in diakinesis into a definite figure, such as was seen in *O. franciscana*, is interesting. It becomes doubly so when we learn that a nearly related plant also shows a definite chromosome grouping, especially when it appears that the grouping in this plant is a different one from that seen in *O. franciscana*, namely, twelve chromosomes arranged in a closed circle, to which is linked a ring consisting of two chromosomes. It emphasizes the suggestion made (1) that perhaps this definite arrangement may be carried back into much earlier stages of the heterotypic prophase, or even into pre-existing cells. In most plants and animals, it is not possible to obtain any evidence regarding chromosome arrangement in this way, since in diakinesis the bivalent chromosomes entirely separate from one another. Thanks to the tendency of the chromosomes to cling together in the species of *Oenothera*, we are enabled to get what may be a hint regarding nuclear construction in a much wider range of forms. It may be that even the position that chromosomes assume with reference to one another in the nucleus may be determined according to fixed rules.

It may be objected that the arrangement here seen represents nothing more than the inability of all but two of the chromosomes to pair, which results in the remaining ones retaining their attach-

ment, and forming a spireme. The uniform presence of the circle of twelve chromosomes, however, strongly suggests an orderly arrangement of the chromosomes among themselves. Furthermore, although all of the chromosomes of the circle look alike, and one cannot be distinguished from another, there is reason to believe that the chromosomes are not scattered promiscuously in the spireme, but are definitely arranged. In the first place, attachment, or at least close approximation of the homologous chromosomes, is the usual condition during diakinesis in plants and animals, whether they have been brought into this relationship telosynaptically or parasynaptically; and we should naturally expect to find in this plant that even though the chromosomes still maintain their position in the spireme, they nevertheless are so arranged that the homologous ones are closely associated. Such a conclusion is also supported by the following considerations.

One can permute the total number of ways in which twelve chromosomes in a circle can be arranged; and, bearing in mind that adjacent chromosomes generally pass to opposite poles in this plant, it is also possible to determine the number of ways in which they can be so arranged that the members of the six pairs will in all cases separate and go to opposite poles. Such a result will be obtained only when homologous chromosomes are adjacent, or are separated from one another by intervals of two or four places. For instance, if chromosome a occupies position no. 1, its homologue a^1 must be in positions no. 2, 4, 6, 8, 10, or 12 in order to be carried to the opposite pole. The result of such computation shows that the ratio of the total number of possible arrangements to those in which the homologous chromosomes are so placed that all of them will separate in the reduction division is 462 : 1.[1] If, therefore, the chromosomes are scattered at random in the circle of twelve, on the average only one cell in 463 will experience an entirely normal reduction division. What is in effect a form of non-disjunction will occur in the other 462 cases, for both members of at least one pair will go to the same pole. In view of the fact that

[1] The twelve chromosomes, acting as units, can be arranged in $\frac{\lfloor 11}{2}$ ways. They can be arranged so that the members of all of the six pairs separate in $\frac{\lfloor 6 \cdot \lfloor 5}{2}$ ways.

about 50 per cent of the pollen grains in this plant are apparently functional, that the percentage of seed fertility is high, and that the resulting plants are probably in general fairly uniform, it certainly does not seem probable that the proportion of typical reduction divisions is so infinitesimal. We conclude, therefore, that the chromosomes are arranged in a definite way. The simplest arrangement, and the one most to be expected from our knowledge of reduction divisions in general, is that in which the homologous chromosomes lie side by side; and I am inclined to believe that such is the chromosome arrangement in *O. franciscana sulfurea*.

GENETICAL

O. franciscana sulfurea differs from *O. franciscana* in being of known hybrid origin. It appeared in the F_2 generation of the cross *O. biennis* × *franciscàna*. The F_1 generation resulting from this cross was strongly patroclinous, and when selfed gave in the F_2 a culture which in general tended to resemble the F_1. One plant appeared, however, which had cream-yellow ("white") flowers, the first *O. franciscana sulfurea*. This plant has been carried on in selfed line for four generations. It has bred true in respect to the color of its flowers, but beyond this it is not possible to say much regarding the nature of the plant from the standpoint of breeding. It undoubtedly exhibits a considerable amount of variation, but the cultures thus far have been so small that a study of this variation has not been possible. There are no breeding data available as yet, therefore, which will answer the question as to whether the strain is pure or hybrid in nature. Regarding the fertility of its pollen, only one test has been made, two plants being examined in 1917. These showed about 50 per cent of abortive pollen grains, which would admit of the possible destruction of large classes of gametes, and the consequent maintenance of uniform cultures in what is really a hybrid stock. On the other hand, the percentage of seed germination is high, so that while *O. franciscana* fulfils closely the tests of a pure species, the situation in *O. franciscana sulfurea* must be regarded as less certain, since its origin and the high percentage of pollen abortion both suggest a possible heterozygosity.

From the genetical standpoint, the chief interest in the cytological work on *O. franciscana* lay in the fact that it showed a perfect regularity of chromosome pairing in diakinesis and the heterotypic metaphase, a situation unusual in species of *Oenothera*. This fact, added to the other evidence, points to the probability that *O. franciscana* B, at least to a large degree, is a genetically pure race.

It is interesting to find that *O. franciscana sulfurea*, although resembling *O. franciscana* closely in most vegetative characters, differs markedly from it in this feature, displaying an almost entire absence of chromosome pairing during diakinesis. One pair only is formed; the other twelve chromosomes are strung together end to end, and form a large closed circle, which lasts until the end of metaphase. The failure to pair is not characteristic of *O. franciscana*, or of other stable species of plants, but is found in most of the unstable species of *Oenothera*, and is also characteristic of interspecific hybrids in general.

If we compare figs. 10–13 with the figures presented by GATES (**6**) for *O. rubrinervis*, and by DAVIS (**2**) for *O. biennis* (an American strain), *Lamarckiana*, and *O. gigas* (**3**), a striking resemblance will be noted. The chromosomes in these plants for the most part are joined together end to end throughout the stage generally known as diakinesis, in a manner much like that seen in *O. franciscana sulfurea*, the failure to show definite pairing being in general characteristic of them. *O. franciscana sulfurea* belongs with these plants, therefore, rather than with *O. franciscana*, in respect to this important cytological peculiarity, the failure of homologous chromosomes to pair in diakinesis.

The regularity of chromosome pairing in *O. franciscana* probably indicates that this plant is largely homozygous in nature. In marked contrast with this situation is the irregularity of *O. franciscana sulfurea*, a condition which may be the result of incompatibility on the part of homologous chromosomes, which would therefore mean that the plant is largely heterozygous. The cytology of other forms not included in the *Lamarckiana* series should be studied, however, and especially of *O. biennis*, one of the ancestral forms of this plant, before this position is definitely assumed as a hypothesis. While the failure of chromosomes to pair

may be said to suggest the possibility of hybridity, therefore, I do not believe that the point is in any sense proved as yet.

Summary

1. *O. franciscana sulfurea* is an evening primrose with cream-yellow flowers, which appeared first in 1914, in the F_2 generation of the cross *O. biennis*×*O. franciscana* B. It has been grown for four seasons in small cultures, and has bred entirely true for the color of its flowers. Except for this characteristic, it resembles *O. franciscana* fairly closely.

2. Archesporial nuclei possess a delicate and copious reticulum of distinct threads, irregularly roughened and thickened with chromatin aggregations, but displaying no parallelism. The identity of the individual chromosomes is entirely lost.

3. The approach of the heterotypic prophase is indicated by an increasing irregularity in the diameter of the threads and of the meshes. This process appears to be the result of the passage of material composing certain threads into others, causing the gradual disappearance of some and the augmentation of others. The threads remain persistently single, only occasional instances of parallelism being found, to which no far reaching significance can be attached.

4. During synizesis, this process continues until the number of threads and meshes has become decreased, and the remaining threads are much thicker than before. The synizetic knot is usually found pressed against the nuclear membrane on the side which is nearest the periphery of the cell. Its position, therefore, seems to be due to the influence of the fixing fluid, which suggests that the synizetic contraction itself is a result of the same cause.

5. Up to this point, the development of the pollen mother cells has paralleled exactly that seen in *O. franciscana*. From now on, however, differences begin to appear, some of which are striking.

6. As the nucleus passes into the "open spireme" stage, the synizetic knot gradually assumes a central position, and begins to loosen. The nucleolus remains plastered against the nuclear membrane. It is filled with an intensely staining substance, probably

chromatin. This material passes into the reticulum by way of one or two prominent threads, which attach the nucleolus to the spireme, and is gradually spread somewhat evenly over the threads as they form the open spireme. The emptying of the nucleolus exposes an intensely staining little endonucleolus.

7. The spireme does not become so evenly distributed through the nucleus as it does in *O. franciscana*. The central portion of the reticulum remains somewhat densely tangled throughout the whole of the open spireme stage. There is no indication whatever of parallel threads, or of a longitudinal split in the spireme at this stage. Chromomeres are clearly present in many cases.

8. The reticulum begins to contract in the center, at the close of the open spireme stage. The peripheral parts are thrown into prominent loops. Condensation may go on to the point where in second contraction nothing appears in the nucleus but an irregularly rounded chromatin mass. More generally, however, the loops do not contract sufficiently to entirely disappear into the central region, and their identity can be traced throughout the whole period.

9. When the second contraction knot unfolds, it can be seen that these loops are a part of a long chain of chromosomes, which are attached end to end. When completely unfolded, the fourteen chromosomes are found to be arranged into a closed circle, consisting of twelve chromosomes, placed end to end; and the remaining two form a pair which at first is linked around the larger chain, but later becomes separated. This arrangement is found in all pollen mother cells at this stage. The nucleolus gradually melts away during this period.

10. The circle of twelve chromosomes is still unbroken upon the disappearance of the nuclear membrane. A multipolar spindle is formed, becoming bipolar. The circle of twelve is brought to the equatorial plate and the chromosomes are so arranged that alternate chromosomes go to the same pole. The remaining pair of chromosomes behaves in the usual manner.

11. The circle of twelve does not break up until early anaphase. Chromosomes are generally distributed equally. In a varying

percentage of cases (averaging 16 per cent) the arrangement of chromosomes in the circle of twelve is upset in metaphase, resulting in six chromosomes going to one pole and eight to the other.

12. Daughter nuclei are reconstituted after the manner described for *O. franciscana* (1), and the subsequent development almost exactly parallels that found in the latter plant. The chromosomes retain their individuality throughout interkinesis. Late in this period a longitudinal split appears, and the two halves of the chromosomes swing apart at right angles, so that each chromosome resembles a maltese cross.

13. The two daughter nuclei pass through the homoeotypic mitosis simultaneously, and are separated into individual cells at the same time. Walls are formed by rapid furrowing, which takes place from without inward. No cell plates are formed.

14. Nuclei with six or eight chromosomes, instead of the usual seven, develop normally as far as they can be traced.

15. The chromosomes seem to be arranged telosynaptically in this plant.

16. The constant grouping of the chromosomes in late prophase into two groups of twelve and two, as well as the generally normal distribution in the first anaphase, suggests the possibility that chromosomes have definite positions with reference to one another in nuclei, and are not scattered promiscuously, and without order.

17. The failure of all but one pair of homologous chromosomes to actually pair in diakinesis suggests the possibility that *O. franciscana sulfurea* is to a large extent heterozygous.

In conclusion, I wish to express my thanks to Professor B. M. DAVIS, for allowing me to collect from his cultures the material upon which this paper is based, and for furnishing the data regarding the history of *O. franciscana sulfurea*, as well as for his continued interest in the work. I am also indebted to the Marine Biological Laboratory, at Woods Hole, Massachusetts, for supplying me with facilities for carrying on the work during the summer of 1922.

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EXPLANATION OF PLATES XIV, XV

All figures were drawn with the aid of a camera lucida, using a Spencer compound binocular microscope, and a Bausch and Lomb 1–12 apochromatic objective with 25X compensating ocular. They have been reduced one-fourth in reproduction; present magnification approximately 2800 diameters.

PLATE XIV

FIG. 1.—Late synizesis: chromatin material beginning to pass from the nucleolus into reticulum; latter much denser than here represented, the drawing being confined as nearly as possible to one level.

FIG. 2.—Slightly later stage.

FIG. 3.—Emptying of nucleolus and passage of material into spireme.

FIG. 4.—"Open spireme" stage.

FIG. 5.—Early second contraction, which begins at center, and peripheral portion thrown into loops; central region may not be as formless and solid as drawing indicates.

FIG. 6.—Later stage; narrow loops.

FIG. 7.—Second contraction about time of maximum contraction; endonucleolus shows clearly.

FIG. 8.—Second contraction knot begins to unfold.

FIG. 9.—Later stage, showing chromosomes arranged telosynaptically into spireme.

FIGS. 10–13.—Spireme completely unfolded, showing arrangement of chromosomes, twelve of them forming large closed circle, and other two a

small ring; in figs. 10 and 13, ring still linked to larger group; in figs. 11 and 12 it has become detached; nucleolus shown in process of melting away in figs. 11–13.

FIGS. 14, 15.—Multipolar spindle; chromosomes still attached.

PLATE XV

FIG. 16.—Typical heterotypic metaphase, showing only the group of twelve chromosomes, still attached, and arranged in regular zigzag fashion, alternate chromosomes headed for same pole.

FIG. 17.—An atypical heterotypic metaphase, showing irregularity in zigzag arrangement, which may result in six chromosomes going to one pole and eight to other.

FIG. 18.—Early anaphase, chromosomes widely separated, but still clinging together; all chromosomes shown, and arrangement regular.

FIG. 19.—Late anaphase, polar view; plates *a* and *b* from same cell, showing unequal distribution.

FIG. 20.—Part of spireme in heterotypic metaphase, showing irregularity in zigzag arrangement.

FIG. 21.—First telophase, just before formation of nuclear membrane.

FIGS. 22, 23.—Late interkinesis, showing split chromosomes, and formation of new nucleoli; one nucleus shows seven, the other eight chromosomes; the two figures from different cells.

FIG. 24.—Homoeotypic metaphase.

FIGS. 25, 26.—Homoeotypic anaphase: fig. 25 shows eight chromosomes in one figure and six in other; fig. 26 shows seven chromosomes in each.

CLELAND on OENOTHERA

CLELAND on OENOTHERA

STUDIES ON THERMOPHILIC BACTERIA

LETHE E. MORRISON WITH FRED W. TANNER

A new interest in thermophilic bacteria has rapidly developed since the investigations of BARLOW (**2**), WEINZIRL (**35**), CHEYNEY (**9**), DONK (**12**), and BIGELOW and ESTY (**4**) have shown their significance in the spoilage of canned foods. The wide distribution of these organisms in nature is shown by the various publications reporting them in water, sewage, snow, canned foods, cotton, hay, manure, soil, etc. Infection of canned foods probably results from the raw materials and water used in the packing process.

History

In a previous paper by MORRISON and TANNER (**21**) a review of the literature dealing with these microorganisms was presented. Since the experimental work reported in the present paper was concerned principally with the thermal relations and thermal death points, table I was prepared to show these relations for the thermophiles which have been described by various investigators.

Experimentation

SOURCES OF CULTURES.—Cultures 53–87 inclusive, used in this investigation, were isolated from samples of soils, and hog and cow feces. The samples of soils were collected from fields which had received different treatments. All of the specimens of soil examined contained thermophilic bacteria. Cultures 1–52 inclusive were isolated from water. Cultures 88 and 89 were isolated from "Ever Fresh Milk," a commercial bottled milk.

METHODS.—Inoculations into different media were made either from 24-hour agar slant cultures or broth cultures. The Descriptive Chart of the Society of American Bacteriologists was used for recording the salient characteristics, and the index number determined under as uniform conditions as possible. Pathogenicity studies were not carried out with all of the strains. One which was used showed no pathogenic properties when inoculated into guinea pigs. This place in the index number was filled in with zero.

TABLE I

Investigator	Organisms described	Source	Temperatures for growth (C.)	Thermal death points of spores (C.)
Flügge (1894).....	Many bacteria, unnamed	Sterilized milk	24°–44° or 27°–54°	Withstood 0.75–5 hours heating at 100°
Rabinowitsch (1895)..	Bacillus thermophilus (eight varieties)	Soil, snow, water, milk, excreta, etc.	34°–75°	Survived steam at 100° for 5–6 hours
Karlinski (1895).....	Bacillus Illidzensis	Hot springs of Illidze in Bosnia	50°–58°	Withstood flowing steam at 100° for 4 minutes
Kedzoir (1896).....	Thermophilic Cladothrix form	River Spree	35°–65°; 35°; opt.	Killed by flowing steam at 100° in 4 minutes
Laxa (1898).....	Clostridium gelatinosum	Füllmasse in sugar manufacture	25°–58°	Not killed by exposure to dry heat at 150° for 15 minutes, or moist heat at 100° for 75 minutes
Tsilinsky (1899).....	Thermoactinomyces vulgaris	Soil	48°–68°; 35°; opt.	Not killed after 20 minutes at 100° in autoclave; spores of second organism killed in 1 minute at 100°; withstood dry heat for 3 hours at 80°
	Thermomyces lanuginosus	Soil	42°–60°; 54°–55°; opt.	
Sames (1900).....	Bacillus I	Earth	56°–70°; opt. 70°–74°; max.	At 750.2 mm. pressure resisted live steam for 3 hours, 10 minutes; spores were formed at 62°
	Bacillus II	Pus from mouse injected with earth and immunized against tetanus	56°–70°; opt. 75°; max.	Resisted steam at 743.7 mm. pressure for 2 hours, 50 minutes; spores were formed at 62°
	Bacillus III	Vaginal mucous during pregnancy	56°–70°; opt. 66°–70°; max.	Resistance of spores formed at 62° at 740.7 mm. pressure = 25 minutes
	Bacillus IV	Raw milk *	50°–60°; opt 66°–70°; max.	Resistance of spores formed at 56° to steam at 746.1 mm. pressure = 15 minutes
	Bacillus V	Aqueous litmus solution	56°–62°; opt. 63°–56°; max.	Resistance of spores formed at 62° to steam at 747.3 mm. pressure = 210 seconds; at 37° at 750.6 mm. pressure = 60 seconds
	Bacillus VI	Same as Bacillus II	56°–70°; opt. 66°–70°; max.	Resistance of spores formed at 62° to steam at 746.1 mm. = 13 minutes; at 37° at 745.3 mm. = 60–70 minutes
	Bacillus VII	Air	50°–60°; opt. 66°–70°; max.	Resistance of spores formed at 56° to steam at 749.3 mm. = 100 minutes; at 749.3 mm. = 60–70 minutes
	Bacillus VIII	Earth	50°–60°; opt. 66°–70°; max.	Resistance of spores formed at 56° to steam at 745.1 mm. = 120 minutes; at 37° at 748.9 mm. = 60–75 minutes
Russell and Hastings (1902).....	Micrococcus form	Pasteurized milk	20°–25°; opt. 76°; max.	Thermal death limit = 76° for 10 minutes
Tsilinsky (1903).....	Bacille no. 7	Infant feces	24°–57°	Resisted heating for 5 minutes at 100° in autoclave
	Bacille no. 8	Infant feces	37°–60° 57°; opt.	Resisted heating for 5 minutes at 100° in autoclave
	Bacille no. 9	Infant feces	42°–45°; opt.	Resisted heating at 100° in autoclave for 5 minutes
	Bacille no. 10	Infant feces	57°; opt. (grew also at 20°)	Resisted heating at 100° in autoclave for 5 minutes
Schardinger (1903)...	Group I, no. 1	Foods, etc.	Room temperature 55°–56°; opt	Withstood 2 hours' exposure to boiling water
	Group I, no. 2	Foods, etc.	Room temperature 55°–56°; opt.	Withstood 2 hours' exposure to boiling water
	Group I, no. 5	Foods, etc.	Room temperature 55°–56°; opt.	Killed within 1 hour in boiling water
	Group I, no. 6	Foods, etc.	Room temperature 55°–56°; opt.	Did not withstand exposure of 1 hour in boiling water

Blau (1906).....	B. cylindricus	Soil	60°–70°; opt.	Thermal death point 100° for 19–20 hours
	B. robustus	Soil	55°–60°; opt.	Thermal death point 100° for 7.5–8 hours
	B. tostus	Soil	60°–70°; opt.	Thermal death point 100° for 19–20 hours
	B. calidus	Soil	60°–65°; opt.	Thermal death point 100° for 7–8 hours
de Kruyff (1910).....	Bacterium no. 1	Soil	37°–70°; 60°; opt.	Killed in 5.5–6 hours in boiling water
	no. 2	Soil	45°–73°; 65°; opt.	Killed at 100° in 6.5–7 hours
	no. 3	Soil	38°–60°; 60°; opt.	Killed at 100° in 6.5 hours
	no. 4	Soil	43°–72°; 63°; opt.	Killed at 100° in 8 hours
	no. 5	Soil	39°–67°; 60°; opt.	Killed at 100° in 6.5 hours
	no. 6	Soil	35°–73°; 65°; opt.	Killed at 100° in 4.5 hours
	no. 7	Soil	35°–70°; 55°; opt.	Killed at 100° in 5.5–6 hours
	no. 8	Soil	39°–70°; 58°; opt.	Killed at 100° in 7.5 hours
	no. 9	Soil	38°–67°; 60°; opt.	Killed at 100° in 7 hours
	no. 10	Soil	38°–68°; 60°; opt.	Killed at 100° in 5 hours
Kroulik (1912).....	Bacillus I, 1	Soil	60°; opt.	Withstood 2 hours' sterilization with flowing steam
	I, 2	Soil	60°; opt.	Withstood sterilization with flowing steam for 2 hours
	II, 1	Soil	30°–68°; 55°–60°; opt.	Resistance greater than that of Bacillus II, 2
	II, 2	Soil	Same as for Bacillus II, 1	Did not withstand action of flowing steam for 2 hours
Negre (1913).....	Bacillus 3, 4, 6	Sands of Sahara	70°; max.; 50°; opt.	Resisted heating at 100° for 15–20 minutes
Bergey (1919).....	Type 1	Dust, contaminated milk media	50°–60°; min. 75°–80°; max.	Thermal death point at 100°, 400 minutes
	2	Dust, soil	37°; min.; 70°; max.	Thermal death point at 100°, 300 minutes
	3	Dust, soil, horse manure	37°–50°; min. 70°–75°; max.	Thermal death point at 100°, 200 minutes
	4	Same as type 3	50°; min.; 75°–80°; max.	Thermal death point at 100°, 180 minutes
	5 var. *a*	Dust, pig feces, horse manure	37°; min.; 60°–70°; max.	Thermal death point at 100°, 120 minutes
	5 var. *b*	Dust, cheese, guinea pig feces	37°; min. 60°; max.	Thermal death point at 100°, 60 minutes
	6	Horse manure	37°; min.; 60°; max.	Thermal death point at 100°, 5 minutes
	7	Same as type 6	37°; min.; 60°; max.	Thermal death point at 100°, 5 minutes
	8 var. *a*	Rabbit's stomach	60°; min.; 70°; max.	Thermal death point at 100°, 120 minutes
	8 var. *b*	Contamination on agar	60°; min.; 70°; max.	Thermal death point at 100°, 120 minutes
	9	Same as type 3	20°–37°; min. 50°–60°; max.	Thermal death point at 100°, 15–60 minutes
Patzschke (1919).....	Streptococcus lacticus thermophilus	Milk	.....	Thermal death point = 75° for 3 minutes; resisted temperature of 85° for 10 seconds
Donk (1920).....	B. stearothermophilus	Canned corn	45°–75° 50°; opt.	Not killed by processing at 118° for 75 minutes
Bigelow and Esty (1920).....	19 thermophilic micro-organisms	.....	.....	Time necessary to destroy known suspension of spores in medium of known H-ion concentration decreases as temperature increases; H-ion concentration influences time necessary to destroy known suspension of spores at given temperature; initial concentration of spores influences time necessary to sterilize medium of known H-ion concentration at given temperature
Grieg-Smith (1921)	Rod-shaped bacterium	Fermenting wattle-bark	.....	Not killed after exposure to 156°–205° for 2.5 hours
Veillon (1922).....	Three anaerobic thermophilic bacteria	Vegetables	20°–58°	Did not develop and killed at about 58°

MEDIA AND TECHNIQUE.—With one or two exceptions, the media used in this study were those recommended by the Committee on the Descriptive Chart (CONN **10**). All media were tested for sterility by incubation at 55° C. for 12–24 hours before use. All of the cultures were grown at 55° C. Due to rapidity of growth it was unnecessary to incubate test cultures longer than 4 days, except in the case of milk cultures, which were incubated for 7–10 days. Two per cent agar was used because it was found to be more suitable for work at 55° than the standard agar. The latter dried out very quickly, becoming unsuitable for growth of bacteria. Broth was found to be a useful medium for the propagation of cultures, since evaporation caused less serious changes in the medium.

MICROSCOPIC FEATURES.—The microscopic features were determined from carbol fuchsin and Gram stains. For staining flagella, the recent method described by PLIMMER and PAINE (**26**) was used with a few slight modifications; it gave very satisfactory results. Agar slant cultures 8–12 hours old were used to furnish the young cells required for flagella staining. Some of the growth was removed and put into tubes containing sterile water which had been held at a temperature of 55° C. These water suspensions were kept at 55° for 30–60 minutes; two or three drops were then placed on slides that had been prepared carefully according to the instructions of PLIMMER and PAINE, and held at 55° for 15–60 minutes. The motility was not disturbed by this treatment, and the flagella were more easily demonstrated by staining. These smears were allowed to dry at 55° over night before fixation and staining.

MISCELLANEOUS BIOCHEMICAL REACTIONS.—As stated, not all of the cultures were used for pathogenicity studies. The one culture which was tested was cultured for 18 hours on fresh rabbit's blood agar slant, from which the growth was removed and suspended in sterile salt solution before injection. Guinea pigs were given injections of this suspension intravenously and intraperitoneally. No pathogenic action was found. BRUINI (**6**) seems to be the only investigator who has attributed pathogenic action to a thermophilic bacterium.

For the determination of gelatin liquefaction the provisional method of the Committee on Bacteriological Technic of the Society

of American Bacteriologists was used. It is designed to distinguish "true liquefiers" (organisms producing ecto-enzymes) from bacteria that produce endo-enzymes that are released from the cell after death and cause liquefaction of the gelatin after incubation of the gelatin after long incubation periods. The cultures were given a preliminary incubation for 24–48 hours in a 1 per cent solution of gelatin at 55° C. After culturing in this gelatin solution, the surfaces of gelatin tubes were inoculated by transferring a drop of the medium. After the incubation period the tubes were cooled to determine whether the gelatin has suffered proteolysis. According to this method all of the cultures except nos. 78, 83, and 84 were found to be gelatin liquefiers. To determine the production of nitrites and gas in nitrate media, both nitrate broth and nitrate agar slants were used.

CARBOHYDRATE REACTIONS.—With certain unimportant modifications, the method of BAKER (1) was used for determining the production of acid and gas for dextrose, lactose, and sucrose. Brom thymol blue was added to these carbohydrate broths before the media were tubed and sterilized. The addition of 15 cc. of 0.04 per cent alcoholic solution of this indicator to a liter of the carbohydrate broth seemed to be about the right concentration of indicator for the detection of acid formation without inhibiting growth. For the determination of diastasic action starch agar plates were used. Dot inoculations were made in the center of the plate instead of the usual streaks.

OTHER CHARACTERISTICS.—Tests for indol were made in nutrient broth and in Dunham's peptone solution. Both the nitrosoindol and Ehrlich's tests were used. Ehrlich's reagent was prepared according to the method described by NORTON and SAWYER (**23**). This test gave more satisfactory results when the tubes were heated slightly. All cultures except no. 81 produced indol.

For the determination of the production of hydrogen sulphide, nutrient broth made with Witte's peptone, over which a strip of lead acetate paper was suspended by means of the cotton plug, was used. The blackening of the paper indicated hydrogen sulphide formation. Streak cultures on "Bacto Lead Acetate Agar" plates were also used. All but cultures nos. 83 and 84 formed hydrogen

sulphide. Litmus milk and sterile milk to which brom cresol purple had been added were used to determine the reactions of the thermophiles in milk. These milk cultures were incubated 7–10 days. Most of the cultures grew well in milk; only two (nos. 54 and 59) produced no apparent change in the milk. Cultures nos. 83 and 84 produced alkali with no other apparent change. The majority of the rest of the cultures showed coagulation and peptonization with an alkaline reaction, some of them having shown a slight preliminary acidity. A few cultures showed slight acidity in milk and a few showed distinct acidity with coagulation, but no digestion of the casein.

TABLE II

Class	Index number	Culture numbers	Numbers of cultures in each class
I.....	5111–01120–1232	1, 2, 4, 6, 9, 10, 13–20, 24, 26, 28, 31–41, 43–52, 55, 56, 86, 88	41
II.....	5211–01120–1232	3, 5, 7, 8, 11, 12, 21–23, 27, 30	11
III.....	5211–01120–1233	25	1
IV.....	5121–01120–1233	29	1
V.....	5111–02120–1232	53, 54, 57, 59–77, 79, 82, 87, 89	26
VI.....	5111–02130–2333	58	1
VII.....	5111–0222o–1232	78	1
VIII.....	5111–01130–2333	80	1
IX.....	5212–01130–2333	81	1
X.....	5111–02230–1222	83	1
XI.....	5111–02230–2222	84	1
XII.....	5121–02120–1232	85	1

INDEX NUMBERS.—The index numbers determined for these strains are shown in table II, where they are grouped into classes. There seemed to be no correlation between the index number and the source of the strains. The organisms in class I differed from those in class II only in the location of the spore. Those in class I had central spores and those in class II had polar spores. Location of the spores is probably a character of minor importance in classification and analysis of bacterial groups. Consequently, the bacteria in I and II may be regarded as belonging to the same class when spore formation, disregarding the location, is considered. BUSHNELL (8) has called attention to the difficulties arising when

only one characteristic is considered, such as location of the spore in the rod. The two classes (I and II) are left separated in this summary, since the separation is called for on the latest Descriptive Chart and because some of the recent reports on anaerobic spore formers indicate this to be a fairly constant characteristic. HALL (15) stated that three distinct groups of anaerobic spore formers could be differentiated on location of the spore: (I) bacteria with central spores which do not swell the rods; (II) subterminal or clostridial spores; and (III) terminal or plectridial spores (*a*, round spores; *b*, elongated spores).

Combining classes I and II, a total of 53 strains is included. This combined class is separated from class V on the basis of relation to combined oxygen. The strains in I and II showed no growth in the closed arm of the fermentation tube in the presence of dextrose. Those in class V showed growth under such conditions. The other classes (III, IV, VI, VII, VIII, IX, X, XI, XII) contain only one strain each, these being separated from the other larger groups on the basis of characteristics of minor importance in systematic studies, such as action on starch, location of flagella, etc. This comparison seems to indicate that the thermophilic bacteria have all of the important characteristics in common.

THERMAL RELATIONS.—Of the 89 cultures discussed in this investigation, 18 were chosen for intensive study of temperature relations and thermal death point determinations. The choice of these 18 cultures was made to include organisms from as many sources as possible. These cultures were grown on agar slants, in gelatin tubes, and on agar plates, at the five different temperatures available for incubation in these laboratories. The agar slants, 5 for each culture, were inoculated by means of a small wire loop from a 24-hour broth culture of the organism to be tested, and these agar slants incubated simultaneously at the five given temperatures. The gelatin was inoculated by placing a loopful of 24-hour gelatin solution culture on the surface of gelatin in tubes. Five tubes were prepared for each organism, and these tubes incubated at the five given temperatures. Large Petri dishes were used for the agar plate cultures, dot inoculations being made in the center of the plate on the surface of the hardened agar. A small wire loop

was used to make these inoculations from 24-hour broth cultures of the organisms.

All of these media were incubated at the five given temperatures for 24 hours in order to have a definite period of incubation; a 24-hour incubation period was used because the agar streak cultures and plate cultures seemed to reach their maximum growth within such a period. The comparative amount of growth attained in

TABLE III*

GROWTH OF THERMOPHILES ON THREE DIFFERENT MEDIA AT FIVE TEMPERATURES (C.)

Culture number	Agar slant					Gelatin					Agar plate				
	25°–30°	37°	42°–45°	55°	60°	25°–30°	37°	42°–45°	55°	60°	25°–30°	37°	42°–45°	55°	60°
1.....	5	4	3	1	2	3	2	1	3	....	4	4	2	1	3
10.....	....	3	2	1	....	4	3	2	1	....	5	4	1	2	3
19.....	4	3	2	1	3	5	2	1	3	4	5	2	1	3	4
21.....	....	4	3	1	2	5	2	1	3	4	5	3	1	2	4
26.....	....	4	2	1	3	....	3	2	1	4	4	4	2	1	3
33.....	5	4	2	1	3	4	3	1	2	....	5	4	2	1	3
37.....	5	4	3	1	2	5	4	3	1	2	4	4	2	1	3
39.....	5	4	2	1	3	....	4	1	2	3	4	4	3	2	1
46.....	....	4	3	1	2	5	4	2	1	3	4	4	1	2	3
47.....	....	4	3	2	1	5	2	1	3	4	5	4	1	2	3
51.....	....	4	3	2	1	5	2	1	3	4	4	3	2	1	2
52.....	....	4	3	2	1	5	2	1	3	4	4	3	1	1	2
53.....	4	3	3	2	1	5	4	3	2	1	4	3	3	1	2
59.....	4	3	3	1	2	....	3	1	2	4	4	3	2	1	3
64.....	....	4	2	1	3	....	3	2	1	4	5	4	3	1	2
67.....	5	3	2	1	4	....	3	2	1	4	4	3	2	1	3
80.....	....	....	3	2	1	....	4	3	2	1	....	....	3	1	2
89.....	....	4	3	1	2	5	4	3	2	1	....	....	3	2	1

* Figures indicate amount of growth, 1 indicating greatest amount of growth at the stated temperatures, 2 the next best, etc.

gelatin and on agar slants at the different temperatures was judged as carefully and as accurately as possible with the naked eye; the diameter in millimeters of the giant colonies produced on the agar plates at these same temperatures was measured.

The data secured are given in table III.

With a few modifications, the method proposed by BIGELOW and ESTY (4) for the determination of thermal death points of typical thermophilic organisms was followed. Nutrient agar slants were inoculated with pure cultures of the organism to be tested,

and grown at 55° for 48 hours. The growth from two agar slants of each culture was brought into suspension by pouring 10 cc. of sterile nutrient broth on to the slants and emulsifying. These suspensions were then transferred to flasks containing 100 cc. of sterile broth and incubated for 7 days at 55° C. At the end of the incubation period, the flasks were placed in a refrigerator for 24–48 hours, when they were heated to 85° for 15 minutes to kill all vegetative forms, cooled immediately, and placed again in the refrigerator to prevent the germination of spores. These were the stock suspensions used for the determination of the thermal death points of the spores. The concentration of spores in the suspensions was determined by plating in different dilutions. The relative ability of the 18 cultures to form spores under these conditions is shown by the counts. The number of spores per cubic centimeter of suspension for the 18 cultures varied from 390 to 73,800,000.

The tubes used for the determinations were hard glass tubes 5 mm. in diameter and 250 mm. in length. They were prepared for use by soaking over night in weak hydrochloric acid solution, rinsing thoroughly with distilled water, draining, wrapping in heavy brown paper in packages of 15 each, and sterilizing. These tubes were inoculated with 1 cc. of the suspensions of spore, sealed off to within 40–50 mm. of the surface of the liquid, and held in the refrigerator until ready to be heated.

The thermal death points to the spores at 100°, 105°, 110°, 115°, and 120° C. were determined by immersing the sealed tubes in an oil bath adjusted to the desired temperature. A DeKhotinsky electric bath containing "Crisco" was used to maintain a constant temperature, and a Wasserman test-tube rack for suspending the sealed tubes in the bath. Before immersing in the oil bath, the temperature was increased one degree in order to compensate for the loss in temperature due to the immersion of the tubes in the oil, and 30 seconds was allowed for the heat to reach the center of the tubes and for the temperature to drop to that at which it was previously adjusted, before recording the time.

A series of tubes was exposed to the desired temperature for definite periods of time, a tube being removed at regular intervals and immediately placed in ice water in order to stop the action of

the heat on the spores. When cold, the tubes were placed in the refrigerator and held until the sterility of the medium could be determined. Sterility tests were made by pouring agar plates. In most cases, if the spores survived the heat treatment, growth appeared in 24 hours. The changes, if any, in hydrogen-ion concentration were followed by the use of Clark and Lub's indicators. Table IV summarizes the work on thermal death point determinations.

TABLE IV

TIME REQUIRED TO DESTROY SPORES OF THERMOPHILES AT STATED TEMPERATURES (C.)

Culture number	Spores per cc.	Time required in minutes for destruction at				
		100°	105°	110°	115°	120°
1.....	3,580,000	110	45	7	2.50	2.00
10.....	390	15	10	3	2.00	1.00
19.....	75,000	40	20	5	2.00	1.50
21.....	5,560,000	150	60	5	2.00	1.50
26.....	8,900,000	80	30	3	2.00	1.50
33.....	27,000	60	50	3	2.00	1.25
37.....	800,000	80	60	4	2.00	1.50
39.....	123,000	190	40	4	2.00	1.75
46.....	40,000	90	50	5	2.00	1.00
47.....	176,000	120	30	5	2.00	1.75
51.....	275,000	70	20	5	2.50	1.50
52.....	73,000,000	60	30	7	2.00	1.25
53.....	184,000	35	25	7	2.00	1.00
59.....	144,000	70	40	7	3.00	1.50
64.....	30,000	70	50	5	2.50	1.50
67.....	1,644,000	90	60	5	2.00	1.25
80.....	500,000	220	70	9	3.00	1.50
89.....	11,000	180	60	9	3.00	1.75

Discussion

The thermophilic bacteria seem to constitute a homogeneous group having the more important characteristics in common. That the function of ability to grow at 55° C. or above may be a relative one is suggested by the results reported in this paper and the work of OPRESCU (**24**), SAMES (**30**), BLAU (**5**), and KOCH and HOFFMAN (**18**), since the conditions seemed to have much influence on growth at the higher temperatures. Table III shows that the organisms grew better at the higher temperatures on agar slants and plates than in gelatin. Some of those which showed no growth at all, or only slight growth, on agar slants after 24 hours incubation

at 25°–30° C. were found to grow well in gelatin at this temperature, causing some liquefaction. The time must be considered in connection with the growth of thermophiles at any temperature. Some strains showed no growth on agar slants at room temperature in 24 or 48 hours, but showed slight growth at this temperature after long incubation. KOCH and HOFFMAN stated that their organisms would not grow in artificial media at 25°–28°, but failed to mention the conditions of incubation. It is possible that statements by several investigators that the thermophilic bacteria would not grow at room temperature may have been made without waiting a sufficient period for growth to appear. The three anaerobic thermophilic bacteria isolated by VEILLON (**34**), however, gave very restricted growth at room temperature even though held for two to three months. RUSSELL and HASTINGS (**29**) and BIGELOW and ESTY (**4**) seem to be the only investigators who have described their technique for determining thermal death points. The results obtained in this investigation are at variance in some respects with the results reported by BIGELOW and ESTY on 19 strains of thermophiles. The strains used by the writers seem to be less resistant than those of BIGELOW and ESTY. Several reasons may obtain to explain this. Their strains were isolated from canned foods which had undergone spoilage, and had thus passed through a selective treatment which would give only strains which were resistant to heat. On the other hand, the strains used in this investigation were isolated from nature, with two exceptions, and had not undergone any such treatment. Slightly different P_H concentrations also obtained in the two investigations. BIGELOW and ESTY report a P_H of 6.0–6.3, while a P_H of 7.4–8.0 obtained in this investigation.

While the initial concentration of spores per cubic centimeter has great influence on thermal death point data, the thermophiles, like other bacteria, vary in their degree of resistance to heat. This is shown by the data in table IV. The suspension of no. 10 contained the least number of spores per cubic centimeter; these were killed by a 15-minute exposure to 100° C. Culture 89 required an exposure of 180 minutes for complete destruction. This difference in the time required cannot be explained solely on the variation in resistance, but apparently is influenced also by the number of spores.

A review of the thermophilic bacteria described in the literature indicates that they have a wide temperature range of growth. In order to express this, different investigators have coined different terms to apply to the bacteria in this group, as follows: thermophiles, orthothermophiles, thermotolerants, obligate thermophiles, true thermophiles, facultative thermophiles, and strict thermophiles. Some divisions have been made on the basis of minimum temperature for growth, others on the basis of maximum temperature, and some on the basis of optimum temperature. The best basis for separation, perhaps, is the optimum temperature for growth. This might allow the following grouping of bacteria according to the temperatures at which they grow best.

I. Strict thermophiles.—Optimum temperature above 55° C.
II. Facultative thermophiles.—Optimum temperature 50°–55° C.
III. Thermotolerant bacteria.—Optimum temperature 40°–50° C.
IV. Mesophilic bacteria.—Optimum temperature 25°–40° C.
V. Psychrotolerant bacteria.—Optimum temperature 10°–25° C.
VI. Facultative psychrophiles.—Optimum temperature 0°–10° C. (?).
VII. Strict psychrophiles.—Optimum temperature below 0° C. (?).

Since the growth of thermophilic bacteria at ordinary temperatures seems to involve a time element, it is probable that canned foods containing thermophilic bacteria might spoil after being stored for a long time at ordinary temperatures. Of course, if stored at higher temperatures it is well known that spoilage occurs very promptly.

Several other problems in connection with thermophilic bacteria have been suggested by the present investigation. These will be taken up in the near future.

Summary

1. Eighty-nine strains of bacteria, studied according to the index number as expressed on the descriptive chart of the Society of American Bacteriologists, fell into twelve classes. All of these separations are based upon unessential characteristics of classification. In the light of this fact, the thermophilic group seems to

be a homogeneous one, the members having in common all of the important characteristics used in classification.

2. A tentative separation of bacteria into groups on the basis of reaction to temperature has been presented.

3. The ability to grow at high temperatures seems to be a somewhat relative function, depending on the conditions of incubation. The so-called strict thermophilic bacteria, however, show a much more rapid growth at 55° C. than at lower temperatures. They must be distinguished from thermo-tolerant bacteria.

4. The thermal resistance of eighteen strains of thermophilic bacteria was studied. At the higher temperatures, 115° and 120° C., the thermal death points fell within narrower time limits than those at the lower temperatures, 100°, 105°, and 110° C.

5. The "index number" is a distinct improvement upon the old "group number," although some of the points in the "index number" would be difficult to determine. For instance, the function of pathogenicity could not be determined without a great deal of experimental work.

6. The character of the media influenced the temperatures for the growth of thermophiles.

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[1] There seems to be some confusion in the literature with regard to the spelling of this investigator's name. The two papers published in the *Annals l'Institute Pasteur* bear the name with two different spellings. The names of some of the other investigators are also found with two different spellings. This accounts for a few mistakes in the names of authors in the previous publication by the present authors.

DILUVIAN FLORA OF POLAND

ANIELA KOZLOWSKA

(WITH FIVE FIGURES)

Introduction

Few geological epochs have been so widely discussed, or made the subject of so many diverse theories, as has the Diluvian epoch. Especially in Europe, glacial formations, as well as their accompanying flora and fauna, have been the subject of many varying theories as to climate and general characters of the epoch. Besides being intensely interesting, this subject is also important in connection with the immediate flora of today.

Two fundamental theories by contemporary students stand out conspicuously. The first and oldest, recognized by a small group of scientists, accepts the existence of only one glacial period, with several thawing periods, causing the appearance of a series of moraines, especially in the central part of Europe. The second theory, that of a series of glacial and interglacial periods, recognized by botanists, zoologists, and archeologists of the old as well as the new world, assumes the existence of several glacial periods, followed by a few successive climatic changes of alternate cold and warm spells (**5, 19**). Although the number of the individual glacial periods for America has been determined (**2**), there are various opinions as to the number in Europe (**7**). It is the aim of contemporary students of glaciology, however, to determine chronologically the number and character of glacial phenomena in both continents. In the present paper, the terminology of PENCK and BRUCKNER (**19**) is accepted as the standard.

Glacial periods in Poland

The first two Alpine glacial periods, Günz and Mündel, did not leave in Europe any definitely ascertained terminal moraines. The third period, Riss, however, has left throughout the whole continent a wall of moraine and many fluvioglacial formations.

In Poland the limit of the so-called "first and largest glacial period" runs through Cracow and Lwow, then turns northward, passing between Wolyn and Podole. This glacier was accompanied by a rich northern tundra, found by ZMUDA (**27**) and SZAFER (**23**), near Cracow and in Krystynopol.

Willows and dwarfed birches, such as *Betula nana*, *Salix herbacea*, and *S. myrtilloides*, were then growing on the outskirts of the glacier. This flora did not last long, however, since the glacier retreating from Cracow caused a rapid succession of changes in the vegetation (**15**). On the treeless tundra there appeared high region trees, such as *Pinus cembra* and *Larix Polonica*. The climate continued to undergo extreme changes. From terrains bordering on Poland, that is, from Germany (**24, 25**), Silesia (**8**), Hungary (**16, 17**), and Russia (**22**), we have several remains of the forest flora, indicating a warmer climate than that of today. It is the so-called second Interglacial in Europe, corresponding to the Alpine Riss-Würm. The next Alpine glacial period, Würm, is as clearly defined in Poland as the first; it reached the upper courses of the rivers Pilica and Bug. In conjunction with this glacial period there were formed younger loess, covering southern Germany, Poland, and Russia. The flora of this period in Poland is known through a very few remains from the caves in Ojców (**13**).

Finally, the fifth and last Alpine glacial period corresponds to the Baltic moraine in Poland, which extends through Prussia and Pomerania (fig. 1). The remains of the flora and fauna in these terrains are not sufficiently abundant or characteristic to serve as data from which to determine positively the climatic changes and the contemporaneous changes in the vegetation of Poland. This problem, being of a general nature, still remains unsolved, and presents many puzzling points, in spite of the numerous fossil remains and the extensive research carried on in European countries and in North America. Possibly the following description, containing a list of the Diluvian flora from the region of Czarna River, found in Poland during the year 1921, may throw some light on the problem.

Fossil flora from region of Czarna River[1]

The Czarna River flows at the southern extremities of the Swiętokrzyskie Mountains on the terrain occupied by the largest glacial period (Riss), and not covered by deposits of the second glacial period (Würm), which reached only as far as the northern

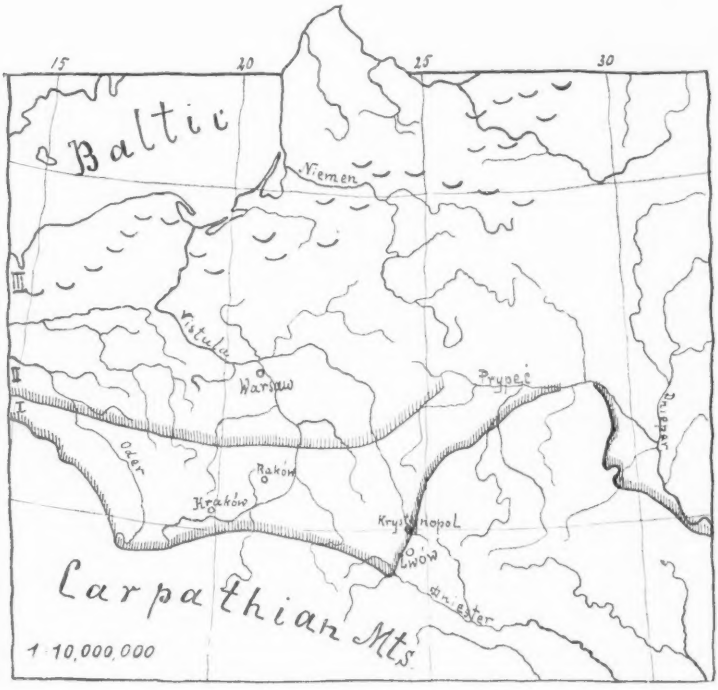

FIG. 1

waterfalls of the Swiętokrzyskie range. The entire shore of this stream is covered by glacial deposits, gravel, sand, and loess. Along its entire course the stream forces its way through these deposits, forming deep ravines, which reveal very interesting sections containing abundant fossil plants.

[1] A more detailed account of the flora found in this region, as well as a thorough description of all the remains, will appear in a Polish publication in the near future.

Directly on the bluish loam, which covers the bottom of the stream, there appear layers one to two meters thick of fluvio-glacial gravel. Above there lies a homogeneous layer of humus, in parts attaining a thickness of one meter. Upon this there stretch indefinitely described strata of diluvian sands. These formations are finally covered by clay, one to three meters thick, resembling in character loess. It lacks, however, the snails typical of loess, such as *Pupa muscorum*, *Helix hispida*, etc.

The humus, dissected by water, showed excellently preserved remains of a forest. It contained leaves with their veins completely preserved, pine needles, seeds, absolutely undamaged, also many fragments of trees, sometimes with the entire roots protruding from the sands. At a width of one kilometer all along the stream the layer of fossilized forest could be detected always in the same stratigraphic position. A number of specimens from various parts, when analyzed, disclosed a homogeneous character of the forest type. In the individual tests, one species or another prevailed only in quantity. The most abundant species of trees were found to be *Fagus silvatica*, *Abies pectinata*, *Carpinus Betulus*, *Tilia platyphyllos*, *Corylus Avellana*, *Pinus silvestris*, *Acer Platanus*, *A. platanoides*, *Betula verrucosa*, *B. pubescens*, *Ulmus* sp., and the herbs *Stachys silvatica*, *Carex* sp., *Vaccinium uliginosum*, *Viscum album*, and *Cirsium* sp.

It is evident from this list that the fossil forest did not vary much in character from that which now covers the hillsides of the Swiętokrzyskie Mountains. The beech and the fir, the commonest of the fossil species, today also compose the greater part of the forests in these regions. Only *Tilia platyphyllos*, one of the now thriving species of our flora, does not grow wild in the Swiętokrzyskie Mountains. Owing to their climatic requirements, these trees are scarce on the southern boundaries of Poland.

What renders this flora peculiar in character, and arouses special interest, is the discovery, although in only one section of the forest, of the presence of loam with several branches of coniferous trees, varying in their physical structure from all the species of evergreens known to the writer in northern Europe. I have made a careful study of this loam. By maceration in water, there

were washed from this loam the following vegetable remains: one-year old branch, several pieces of two-year old roots with many one-year branches, a few pieces of three and four-year old wood, about 20 cm. long with well preserved bark, all belonging to the previously mentioned coniferous trees. Besides, there were one pine needle, with its structure unfortunately disarranged, about a dozen seeds of reed grass, fragments of roots and leaves of grass and reed grass not definitely described, fruits of hazelnut and beech, a few pieces of wood of the fir and elm, single leaves of moss, and very many spherical objects of diluvian age known as *Cenococcum geophillum*. The appearance of all this material was the same as of that lying in the direct vicinity of the forest. All of these remains disclosed in their structure an absolutely young character, and did not show any traces of the action of water. The one and two-year old branches of coniferous trees had falling bits of bark preserved on their exterior, also tiny gnarls on the side branches, and no traces of having been surrounded by water. Moreover, the delicate leaves of moss and reed grass, simultaneously found, apparently present sufficient proof that this material was not subsequently thrust among the forest humus, but that it represents the original composition, and therefore is contemporaneous with the forest described.

Cross-sections of the branches and roots of coniferous trees, obtained by cutting with a razor, and not treated with any chemicals, revealed a well preserved structure of wood and bark, and permitted the exact tracing of the anatomical structure. The three-year old branches showed the following structure: (1) Lack of resin canals in the wood, in the longitudinal and diagonal cross-sections. (2) Lateral walls of the uniseriate ray cells with simple pits. (3) Tracheids of the wood in a radial cross-section reveal a rather changeable structure. In the autumn wood and partly in the summer wood there is but one row of bordered pits, whereas in the spring wood tracheids of slightly larger dimensions disclose bordered pits arranged in two rows. Transitional forms are met with occasionally; in one and the same tracheid, the pits place themselves either singly or doubly. (4) Resin cells of a dark brown hue, scattered along the summer wood. These characteristics establish

beyond doubt the relationship of this wood to the Abietineae type, therefore to one or another of the genera *Abies*, *Pseudolarix*, *Cedrus*, or *Tsuga*.

JEFFREY (**11a**) showed what significance the study of diagonal cross-sections of young branches and roots has for the Abietineae type. The appearance and number of resin canals in the individual species are characteristic of this group. Three genera, *Abies*, *Pseudolarix*, and *Cedrus*, have distinct resin canals in the original wood, and therefore in the pith of the one-year old branches; besides they have many resin canals in the bark. The young roots disclose a similar structure; one sap duct in the original wood and many in the texture of the bark. *Tsuga* alone varies fundamentally in this respect, having only one sap canal in the original wood of the root. There is an entire lack of these canals in the bark and the pith of the branches.

The fossil wood examined showed, in diagonal cross-sections of the roots and branches, a structure corresponding exactly with that of *Tsuga* as described by JEFFREY. A thick layer of bark, surrounding the branches and the roots, does not possess resin canals. It is composed of homogeneous dead cells of the bark texture, which cells are divided by sclerenchyma filaments. Similarly, the original wood of the branch does not show any resin canals; they appear only in the pith of the one-year old root. These characters indicate that this wood belongs to *Tsuga*.

Other characteristics of *Tsuga* have been noticed by PENHALLOW (**20**). In his opinion the disposition of the resin cells on the exterior part of the summer wood is extremely characteristic of *Tsuga*. In wood as young as the observed branches the disposition of the resin cells does not appear in such a typical way, but numerous dark cells are aggregated on the exterior of the spring wood. Finally, a character determining the genus with certainty is the disposition of the bordered pits in the tracheid of the wood. In this genus of the type Abietineae, the species *Tsuga canadensis* exclusively has in its spring wood bordered pits disposed in double rows. This character is typical of our fossil wood, and has been verified in all the cross-sections made by the writer. The discovery of the American species, *Tsuga canadensis*, among the mixed wood

characterizing central Europe, throws an important light, first on the determination of the age of the fossil flora of Rakow, and second on the floral history of the glacial period.

As I noticed before, the fossil wood in the Czarna River section lies upon a substratum of stones and gravel, and underlies glacial sands and loam. As this stratum is situated not far from the terminal moraine of the first glaciation (Riss), the stones and gravels appearing at the bottom of the streamlet must be remnants of that glaciation. They probably correspond to the lower moraine. The fossil forest with *Tilia platyphyllos* and the *Tsuga canadensis*, showing climatic conditions milder than today, has been able to grow only after the complete retrogression of the glacier near Cracow. This wood, therefore, will correspond to the interglacial period, called in the Alps Riss-Würm. The next glaciation (Würm), stopping in Poland at the beginning of the rivers Pilica and Bug, has covered with sand and loess the rising of Malopolska. The sand and loam with loess character which cover our fossil wood correspond to that second glaciation.

Interglacial flora of Rakow and other discoveries in Europe and America

In all central Europe, and especially in Germany, Denmark, and Switzerland, we know of a fossil flora very abundant and rich in the number of species from the interglacial period. Many times the exact determination of these fossils has presented great difficulties, allowing various interpretations of the same section by the investigators. It can only be stated with certainty that the numerous fossils of the warm diluvial flora in Europe must be scattered between various interglacial periods. The best known of these interglacial periods is the Riss-Würm, which occurred in Europe between the first and second glaciation.

The essential trait of each of these fossil floras is the appearance among the mixed leaf forests of central Europe of some remnant of the flora corresponding to a warmer climate of south Europe. Among such plants that may be considered as typical in the interglacial period are *Buxus sempervirens* and *Ilex aquifolium*, which have been found in Switzerland (**26**), Germany (**25**), and France

in the north, far from their present area of distribution. Besides this we have a quantity of other species of similar character, known from occasional discoveries. Thus there have been found in France *Acer opulifolium, Laurus canariensis, Cercis siliquastrum, Ficus carica, Evonymus latifolius, Juglans regia* (**6**); in Hungary in Ganöcz and Lueski (**17, 18**) *Cotinus Coggyria, Astragalus hamosus;* in Russia near Oka River *Fagus silvatica,* all of which species have been discovered far from their present limits. A similar phenomenon of the advance of the southern species to the

FIG. 2.—Distribution of *Brasenia purpurea Mich.*

Recent
Fossil

north in the interglacial periods has been observed in America. In our fossil flora from Rakow a plant of such a type is *Tilia platyphyllos*, known from many interglacial periods in Europe, which indicates more favorable climatic conditions than the present.

Besides these plant species, there are several plants from the interglacial periods of special interest. These are species which do not exist any more in Europe, but are still found in other parts of the world. *Brassenia purpurea* belongs to these. Fig. 2 gives a view of the distribution of this plant. It originated in North America, where it covers the lakes and rivers from Canada to Mexico; it appears in two places in Africa; it is known as an

extremely rare plant in Australia, in the south of Japan, in India, and in Manchuria. In Europe it is no longer in existence, but it has been found in almost all the exposures of the interglacial flora in Germany (14), Denmark (1), Switzerland, and Russia. This widely distributed but scattered species, appearing in several continents, proves that we are dealing with an ancient type, which

FIG. 3.—Distribution of *Dulichium spathaceum*

existed perhaps in the Tertiary period on all of the continents, and today remains only in certain isolated areas. As this species is known in Europe from the Tertiary period, we can assume that in our diluvial epoch it is the last remnant of the abundant Tertiary flora which was almost entirely exterminated during the glaciation. The same may be said of *Nymphaea Lotus*, existing today in Egypt and discovered by PAX (17) in Ganöcz, Hungary.

In America, in spite of the comparatively limited studies on the diluvial flora, six species of plants are known from the interglacial period, which are not in existence today, and which are remnants of the Tertiary period (**3, 4, 11**). Other plants of limited geographic distribution are two species entirely American and found in the European diluvium. One of these is *Dulichium spathaceum*, which today is confined to eastern North America, but which was

FIG. 4.—Distribution of *Fraxinus Americana*

an element in the flora of Europe (**9**) in the interglacial period, in Denmark, and in Lauenburg on the Elbe (fig. 3). Moreover, this species is not known in the European Tertiary period. The other species is *Fraxinus americana*, somewhat similar in its distribution to *Dulichium*, limited today to eastern North America. In Europe it has been found in Thuringe by SCHRÖTER (POHLING **21**), and is illustrated in fig. 4. *Tsuga canadensis* would represent the third species of similar distribution. It is a northeastern American

species, and does not appear in our existing flora anywhere else (fig. 5).

ENGLER (5) discusses the floral relationship between America, Asia, and Europe on the ground of comparative lists of plant associations of the three continents, and concludes that in the Tertiary there must have been a continental connection between Asia and America, and that migration of the species took place by

FIG. 5.—Distribution of *Tsuga Canadensis*

means of that connection. The lack of identical types in eastern America and western Europe which were not known in Asia (ENGLER quotes only ten types), and the depth of the ocean between Great Britain and Greenland, prove in the opinion of ENGLER a very ancient severing in this region of the two continents. On the other hand, he admits the possibility of a continental connection between America and Europe through Greenland, Spitzbergen, and Nova Zembla. The very rich Miocene flora in Greenland and Spitz-

bergen, as described by HEER (10), shows great analogy; the coniferous woods abundantly represented today in North America prevailed there. With the advent of the glacial period all of the species from the boreal region were driven south before the advancing glacier.

In the Miocene flora of Spitzbergen, among the numerous conifers, five species of the *Tsuga* type were discovered, which are not known either in the European or in the American Tertiary period (12). Because of the entire lack of discoveries it is difficult to assume with confidence that this genus did not exist in that period either in Europe or in America. One may assume with a certain degree of probability, however, that the *Tsuga canadensis* discovered near Rakow is a circumpolar species of ENGLER, which in the beginning of the glacial period migrated from Spitzbergen and Greenland to eastern America, and through Nova Zembla has also reached Europe. Whether the connection between America and Europe known only from a few examples can be explained in the same way, remains a problem to be solved by further investigations.

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RELATION OF SEED WEIGHT TO GROWTH AND VARIABILITY OF WHEAT IN WATER CULTURES[1]

SAM F. TRELEASE AND HELEN M. TRELEASE

During the recent development of water culture experimentation, the methods employed in the study of the mineral requirements of plants have been improved rather rapidly. The general method is necessarily so complicated, however, that many of its details still remain to be worked out and perfected. One of the greatest difficulties in the interpretation of the results of water culture studies arises from the high degree of variability of the plants. Variability may obscure the relations between the growth of the plants and the physiological values of the solutions tested. Recently it has become increasingly evident that, to reduce the disturbing influence of plant variability, relatively large numbers of plants must be used in each of the several cultures that are to be compared, and statistical methods must be employed as an aid in the interpretation of the results of such studies (BRENCHLEY **2, 3**; STILES **12, 13**; STILES and JÖRGENSEN **14**; DAVIS **4**).

One of the important questions in the general water culture method, therefore, is that of the number of plants needed to secure reliable results. In order to bring the work into conformity with the necessarily limited time and facilities at the disposal of any one experimenter, it is important from a practical point of view to consider methods by which variability may be limited and the number of plants to be grown in each culture may be as small as possible.

To limit the range and degree of variability, investigators have generally used seedlings that appear to be nearly alike in size and general vigor; some workers have used seeds of a pure line, and a few have even employed seeds selected for uniformity in weight (BRENCHLEY **2, 3**). It might be anticipated that such precautions would tend to reduce the range of variability in the plants, but

[1] Botanical contribution from the Johns Hopkins University, no. 76.

further work seems desirable, to determine quantitatively, if possible, just how effective these methods of selection may be. Although it is known that seed weight may have a pronounced influence on plant yields (KIESSELBACH and HELM 7), the importance of this appears not to have been emphasized sufficiently in the literature of the water culture method. It was with the idea of securing further information on this question that the present study was undertaken. This paper reports experiments dealing with the growth and variability of young wheat plants, as related to the original seed weight. It aims to emphasize the importance, in water culture technique, of employing seeds that have been selected for uniformity of original weight.

The experiments were carried on at the laboratory of plant physiology of the Johns Hopkins University. It is a pleasure to acknowledge indebtedness to Professor B. E. LIVINGSTON for suggestions in the preparation of this paper.

Experiments and results

The seeds used in this study were from a pure line of Marquis wheat (Marquis, Saskatchewan, no. 70, Selection no. 313), grown near Saskatoon during the summer of 1922. They were supplied by the Department of Field Husbandry of the University of Saskatchewan, through the courtesy of Professor MANLEY CHAMPLIN. In table I is shown the frequency distribution of seed weights, as shown by a random sample of 300 seeds from this lot.

Four groups of seeds were selected on the basis of weight. The first or light group included seeds weighing less than 24.5 mg. The second or medium group included seeds weighing more than 32.5 mg. and less than 36.5 mg. The third or unselected group included seeds taken by "random sampling" from the general stock. The fourth or heavy group included seeds weighing more than 40.5 mg. To expedite the work of securing seeds to represent each of these groups, a sorting by appearance was first made; then the weight group to which each individual seed belonged was determined by means of a balance, and those having the desired weights were saved.

The data for the seeds actually used are presented in table II. The second and third columns present data based on the frequency

distribution of weights, derived from table I, the second column showing the ranges of seed weights included in each group, and the third column giving the percentage of seeds falling in each group.

TABLE I

FREQUENCY DISTRIBUTION OF SEED WEIGHTS FOR MARQUIS WHEAT GROWN AT UNIVERSITY OF SASKATCHEWAN DURING SUMMER OF 1922, BASED ON WEIGHINGS MADE OCTOBER 26–30, 1922

WEIGHT CLASS (MG.)	FREQUENCY		WEIGHT CLASS (MG.)	FREQUENCY	
	Actual	Percentage		Actual	Percentage
18	2	0.7	33	22	7.3
19	0	0.0	34	17	5.7
20	1	0.3	35	28	9.3
21	0	0.0	36	25	8.3
22	2	0.7	37	16	5.3
23	4	1.3	38	15	5.0
24	5	1.7	39	19	6.3
25	12	4.0	40	16	5.3
26	3	1.0	41	15	5.0
27	13	4.3	42	8	2.7
28	5	1.7	43	8	2.7
29	14	4.7	44	5	1.7
30	13	4.3	45	3	1.0
31	16	5.3	46	1	0.3
32	12	4.0			

TABLE II

DATA FOR SEEDS OF MARQUIS WHEAT INCLUDED IN EACH WEIGHT GROUP EMPLOYED IN CULTURES

GROUP	DATA BASED ON FREQUENCY DISTRIBUTION OF WEIGHTS		DATA FOR SEEDS CHOSEN FOR EXPERIMENT		
	Inclusive range of weights in group (mg.)	Percentage falling in group	Number chosen	Mean original weight (mg.)	Mean dry weight* (mg.)
Light	18–24	4.7	245	19.3	17.2
Medium	33–36	30.6	281	34.8	31.0
Heavy	41–46	13.4	231	44.5	39.6
Unselected	18–46	100.0	250	33.5	29.8

* Calculation based on determinations of moisture content of similar seeds from same lot.

The third column shows that, if one hundred seeds were taken at random, about five would fall in the light group, about thirty-one in the medium group, and about thirteen in the heavy group. The last three columns of table II give data for each group of

seeds as actually employed in the experiment. The fourth column shows the number of seeds chosen to represent each group, the fifth column shows the mean original weight of these seeds, and the sixth column shows the mean dry weight of the seeds (based on determinations of moisture content made with similar seeds from the same lot). Eighty seedlings were selected at random from the original lot obtained from the light seeds, and these were divided into two sets, each set being grown in one of the two culture vessels for that group. Corresponding duplicate sets, each containing forty plants, formed the cultures for the medium, unselected, and heavy groups.

The same general methods were employed in this study as were used by TOTTINGHAM (**15**), SHIVE (**11**), TRELEASE (**16**), and other workers. All cultures were in the same solution, which was one productive of good growth. The total concentration corresponded to about 0.5 atmosphere of osmotic pressure at 25° C. The solution contained the following partial volume-molecular concentrations of its constituent salts: 0.00525 M KH_2PO_4, 0.00189 M $Ca(NO_3)_2$, 0.00336 M $MgSO_4$, and 0.00001 M $FeSO_4$. For the three main salts, these partial concentrations correspond to relative molecular proportions of 50 KH_2PO_4, 18 $Ca(NO_3)_2$, and 32 $MgSO_4$.

The culture vessels were 3-gallon, glazed earthenware "butter" jars. Each jar was filled with solution and covered with a paraffined Portland cement top with eight circular openings. The seedlings were mounted in cork stoppers in the usual way, the stoppers being held in the openings in the cement top. Each stopper bore five seedlings, forty seedlings for each of the two culture vessels in each set. The cultures were placed near the margin of a rotating table, so that all of the plants were exposed to approximately the same fluctuations in environmental conditions throughout the period. The solutions were renewed every five days, thus giving approximately the renewal rate suggested by LIVINGSTON (**8**), each plant being supplied with an amount of solution corresponding to about 53 cc. per day.

The seedlings were grown from November 4 to December 15, 1922, a period of forty-one days. During the first two days the seeds were germinating on wet blotting paper in a damp chamber,

and during the next four days the seedlings developed at the surface of tap water, being supported on a netting germinator. The plants were then transferred to the culture jars, in which they grew for thirty-five days. While the plants were growing in the culture solution, the average daily maximum temperature was 26° C., and the average daily minimum was 18.1° C. During this period the corrected evaporation from a Livingston white spherical porous cup atmometer gave a daily mean of 16.3 cc. At the end of the culture period the tops of the eighty individual plants of

TABLE III

MEAN YIELD OF TOPS, ROOTS, AND WHOLE PLANTS FOR WHEAT SEEDLINGS GROWN FOR FORTY-ONE DAYS (NOVEMBER 4 TO DECEMBER 15, 1922)

GROUP	TOPS		ROOTS		WHOLE PLANTS	
	Actual (mg.)	Relative*	Actual (mg.)	Relative*	Actual (mg.)	Relative*
Light	83.4	1.00	10.5	1.00	93.9	1.00
Medium	126.7	1.52	16.3	1.55	143.0	1.52
Unselected	129.5	1.55	16.4	1.56	145.9	1.55
Heavy	143.0	1.71	17.3	1.05	160.3	1.71

* Each relative value shows weight in terms of the weight for the corresponding light group taken as unity.

each group were harvested separately and dried to an approximately constant weight at 102° C. The top dry weight of each individual plant was then determined. The roots of all the eighty plants in a group were harvested together, and their total dry weight was determined. A summary of the results is given in tables III and IV.

Discussion

The data of table III, giving the mean yield of wheat seedlings produced from the seeds of each of the four groups, show that these yields are roughly proportional to original mean seed weights. This statement holds true for tops, roots, and whole plants. It is important to ask whether the observed differences among the mean yield values are significant of true differences between the several plant populations with respect to their final dry yields. To determine whether the difference between any two means is significant,

the difference is to be considered of course with respect to its own probable error. Data for this consideration are available for top yields only (table IV), individual weights of roots not having been secured. For mean top yield, the difference between the light and medium groups is 43.3 mg.; this difference amounts to 27.1 times its probable error ($43.3 \div 1.6 = 27.1$). Since the odds are overwhelmingly against the occurrence of a deviation as great as or

TABLE IV

TOP YIELD DATA FOR WHEAT SEEDLINGS GROWN FOR FORTY-ONE DAYS (NOVEMBER 4 TO DECEMBER 15, 1922)*

Group	Mean top yield in mg. (M)	Standard deviation in mg. (σ)	Probable error of single observation in mg. (E_s)	Probable error of single observation, as percentage of mean (P_s)	Percentage coefficient of variability (C)
Light	83.4 ± 1.3	17.7	11.9	14.3	21.2 ± 1.2
Medium	126.7 ± 1.0	13.5	9.1	7.2	10.7 ± 0.6
Unselected	129.5 ± 2.1	27.4	18.5	14.3	21.2 ± 1.2
Heavy	143.0 ± 1.5	19.5	13.2	9.2	13.6 ± 0.7

*The statistical calculations were made by means of the following standard formulas: Mean $(M) = \frac{\Sigma w}{n}$, Σw being the sum of the individual top weights, and n the number of variates (80). Standard deviation $(\sigma) = \sqrt{\frac{\Sigma v^2}{n}}$, Σv^2 representing the sum of the squared deviations of each weight from the mean, and n the number of variates (80). Probable error of a single observation $(E_s) = \pm 0.6745 \times \sigma$, in which 0.6745 is the usual statistical constant, and σ is the standard deviation. Probable error of the mean $(E_M) = \pm \frac{E_s}{\sqrt{n}}$, E_s being the probable error of a single observation, and n the number of variates (80). Probable error of a single observation, this error expressed as percentage of the mean, $(P_s) = 100 \times \frac{E_s}{M}$, in which E_s represents the probable error of a single observation, and M the mean. Coefficient of variability $(C) = 100 \times \frac{\sigma}{M}$, σ representing the standard deviation, and M the mean.

greater than 27.1 times the probable error, it may be concluded that the plants of the medium group really differ from those of the light group by an increase in top yield of about 43.3 mg. In the medium and unselected groups, it is not likely that the difference between the top yield data for these groups is significant, since the observed difference of 2.8 mg. is only 1.2 times its probable error ($2.8 \div 2.3 = 1.2$). The mean seed weights were nearly the same for these two groups, and the top yields do not differ significantly. The next difference, however, that between the yield for the unselected and that for the heavy group, appears to be surely significant, since the

difference of 13.5 mg. is 5.2 times its probable error (13.5÷2.6 = 5.2), the odds being several thousands to one against the occurrence of a deviation equaling or exceeding 5.2 times the probable error.

The evidence is very strong, therefore, that the mean top yields are directly related to original seed weights, the heavier seeds producing higher yields. Moreover, it seems reasonable to conclude that the data for root yields are reliable in indicating a similar general relationship between original seed weight and final yield. The relationship is clearly indicated by the actual data for yields of roots and of whole plants (roots plus tops), and there seems to be no reason for regarding it as less definite for these than for top yields.

The relative values in table III show the degree of influence that original seed weight may exert upon yield at the end of forty-one days of growth. If the mean top yield of the light group is considered as having a relative value of 1.00, then the mean top yield of the medium group has a value of 1.52, that for the unselected group (the original mean seed weight for this group being also medium) has a value of 1.55, and that for the heavy group has a value of 1.71. For root yields the corresponding relative mean values are 1.00 for the light group, 1.55 for the medium group, 1.56 for the unselected group, and 1.65 for the heavy group. For yields of whole plants (top plus roots) the corresponding relative values are the same as for top yields. The close agreement between the relative mean values for top and root yields in each group appears to furnish evidence for the proposition that the mean root yields obtained are probably about as reliable as the mean top yields, in spite of the fact that no statistical evidence for root yields is available here. Furthermore, this agreement indicates, of course, that seed weight may be expected to influence both top and root yields in a similar manner and to a similar extent.

Special attention was not given to the relationship of top length in the very young seedlings to growth or variability of the plants at the end of the culture period. Some evidence in this connection was obtained, however, from the measurements of top length, which were made for all seedlings at the end of six days' growth, when the seedlings were transferred from the netting germinators

to the culture solutions. These measurements of top length are shown in table V, together with the mean dry weight values for the seeds. These data at least suggest that top length was directly related to initial seed weight.

The preceding discussion has emphasized the pronounced influence which heavy seeds, as compared with light seeds, may have on the dry weight at the end of a growth period of forty-one days. The absolute values for the final yields are clearly greater with seeds of greater weight. It is interesting to note incidentally that the relative increase of dry material, expressed in terms of initial seed weight, was smaller for the heavier seeds than for the lighter seeds. To show this more clearly, data indicating the growth rates for the culture period are presented in table VI.

TABLE V

MEAN DRY WEIGHT OF SEEDS AND MEAN LENGTH OF TOPS OF SEEDLINGS AFTER SIX DAYS' GROWTH

GROUP	SEEDS		TOPS	
	Mean dry weight (mg.)	Difference (mg.)	Mean length (mm.)	Difference (mm.)
Light	17.2	0	88.3±1.1	0
Unselected	29.8	12.6	101.1±0.7	12.8±1.3
Medium	31.0	1.2	102.8±0.8	1.7±1.1
Heavy	39.6	8.6	107.8±0.6	5.0±1.0

Each of the two indices, the ratio of increase to seed weight and the efficiency index, has particular characteristics that must be borne in mind when it is used as a general measure of the rate at which new material accumulates in the plant. In general, the values of each may be expected to vary with the stage of development of the plant. Without going into these considerations in detail, the data in the last two columns of table VI indicate that the seedlings of the light group increased in weight more rapidly, with respect to the original seed weight, than did those of the medium or those of the unselected groups, which in turn increased relatively more rapidly than did the seedlings of the heavy group. It is apparently safe to conclude that, although greater seed weight resulted in greater final product, the heavier seeds did not yield

as great a relative increase, in proportion to their initial weight, as did the lighter seeds.

Since the initial weight of the seeds may have a pronounced effect upon the amount of material produced by the plant in its early stages of growth, it follows, of course, that seeds which are uniform in weight should produce plants which exhibit only a narrow range of variation in dry weight. That plants showing a relatively low degree of variability may be secured by employing seeds that have been selected for uniformity in weight is clearly shown by the present tests. This may be observed from table IV, by comparing the

TABLE VI

GROWTH RATE AS INDICATED BY ACTUAL INCREASE IN DRY WEIGHT, RATIO OF INCREASE TO DRY WEIGHT (SIMPLE INTEREST RATE), AND THE EFFICIENCY INDEX OF SEEDS (RATE OF CONTINUOUSLY COMPOUNDED INTEREST)

Group	Mean original dry weight (mg.)	Mean increase of dry material* (mg.)	Ratio of increase to seed weight†	Efficiency index of dry weight production‡
Light	17.2	76.7	4.46	1.70
Medium	31.0	112.0	3.61	1.53
Unselected	29.8	116.1	3.90	1.59
Heavy	39.6	120.7	3.05	1.40

* Final dry weight of whole plant minus dry weight of seed.

† Increase divided by dry weight of seed; "simple interest" rate for the 41-day period.

‡ Natural logarithm of final dry weight of whole plant minus natural logarithm of dry weight of seed; representing rate of "continuously compounded interest" for the 41-day period.

statistical data for the medium group with those for the other groups, particularly with those for the unselected group. It is important to remember that only in the medium group were the seeds chosen in such a way as to secure a low range of variation in weight. In the medium group all of the seeds were selected so that their weight lay within a range of 4 mg. (32.5–36.5 mg.), while the unselected seeds had a range in original weight of about 28 mg. (about 18–46 mg.). Table IV shows that the plants of the medium (selected) group had a coefficient of variability of only 10.7 ± 0.6 per cent, while those of the unselected group had a corresponding coefficient of 21.2 ± 1.2 per cent. The difference

may safely be regarded as significant, for it amounts to 8.1 times its probable error ($10.5 \div 1.3 = 8.1$). Thus selection of seeds for uniformity of original weight rendered the variability of the plants grown from them markedly less than it would have been without such seed selection.

To illustrate the value of seed selection as a procedure in water culture technique, it may be of interest to consider the number of plants that would probably have been needed in each culture in order to obtain a certain desired degree of statistical accuracy, and to compare this number for plants from selected seeds with the corresponding number for plants from unselected seeds. A calculation of the required number of plants may be made by rather simple statistical formulas that are commonly used in connection with chemical analysis of variable samples, such as of fruits and soils (DENNY **5**, HAYNES and JUDD **6**, ROBINSON and LLOYD **10**, WAYNICK **17**, WAYNICK and SHARP **18**).

How many plants would have been required in each of two cultures in different nutrient solutions, the plant populations having the same known degree of variability, in order that there might be odds of 31.36 to 1 that a certain percentage difference between the two culture means is indicative of a significant difference between the nutritional values of the two solutions used? The formula here employed may be written: $N = 2 \times (3.2)^2 \times (P_s)^2 \div D^2$, in which N represents the number of plants required, 3.2 is obtained from a table of odds (PEARL and MINER **9**) and corresponds to odds of 31.36 to 1, P_s is the probable error (expressed as percentage of the mean) of a single plant weight, and D is the percentage difference. This formula was used in making the calculations for table VII, which shows the number of plants required in each of two cultures in order that various percentage differences between the two culture means might be considered significant, with an assurance of 31.36 to 1 odds.

In examining the data of this table, the figures shown in the fourth column may be considered first. For the medium group, that is, for the group with seeds selected for uniformity in seed weight, the number of plants required is shown as eighty, which, it will be remembered, is the number of plants actually used in each

group. The item for the unselected group shows that it would require 316 plants from unselected seeds to obtain the degree of accuracy secured with the eighty plants from selected seeds. It will be observed that 316 plants would also be required for the light group, and that an intermediate number, 131, would suffice for the heavy group; but it is to be remembered that neither in the light group nor in the heavy group were the seeds really selected for uniformity, those in the light group being chosen merely to have weights below 24.5 mg., and those in the heavy group to have weights above 40.5 mg.

The last column in table VII, which gives the relative number of plants of each group that would be required for a certain degree of

TABLE VII

NUMBER OF PLANTS NEEDED IN EACH OF TWO CULTURES IN ORDER THAT VARIOUS PERCENTAGE DIFFERENCES BETWEEN THE TWO CULTURE MEANS MAY BE INDICATIVE (WITH ODDS OF 31.36 TO 1) OF SIGNIFICANT DIFFERENCE BETWEEN THE TWO MEANS

Group	Percentage difference					Relative number of plants required for each group
	1	3	3.64	5	10	
Light	4188	465	316	168	42	3.95
Medium	1062	118	80	42	11	1.00
Unselected	4188	465	316	168	42	3.95
Heavy	1733	193	131	69	17	1.63

statistical accuracy, shows that 3.95 times as many plants would be needed with the unselected group as would suffice with the medium, or selected, group.

The other columns in table VII show the number of plants for each group that would be needed for various degrees of accuracy. The table thus illustrates how the required number of plants changes with the degree of accuracy desired, this number being inversely proportional to the square of the difference (D). For example, the table shows that if the plants exhibit the variability of the medium seed group, 1062 plants should be needed for a 1 per cent difference to be regarded as significant, 118 plants should be needed for a 3 per cent difference to be significant, forty-two plants should

be needed for a 5 per cent difference to be significant, and eleven plants should be needed for a 10 per cent difference to be significant. The data of table VII thus serve to illustrate in an emphatic way the main point brought out by the present study. They show that the employment of seeds selected for uniformity of original weight may enable the experimenter to secure reliable results with a much smaller number of individual plants than would be required if unselected seeds were used.

Conclusions

1. The yields of both tops and roots were found to be directly related to original seed weight; light weight seeds gave low yields, medium weight seeds gave medium yields, and heavy weight seeds gave high yields.

2. From the relation between seed weight and yield, it follows that seeds which are nearly uniform in weight should produce plants which exhibit a relatively narrow range of variation in weight, and selection of seeds for uniformity of original weight rendered the variability of the plants grown from them markedly less than it would have been without such seed selection.

3. Top length in the very young seedlings is directly related to initial seed weight, and therefore selection of young seedlings for uniformity of height may exert a similar, although probably less pronounced, influence in making the variability small.

4. Although the yields from heavier seeds were higher than the yields from lighter seeds, the differences in yields were relatively smaller than the differences in seed weight; greater seed weight did not result in a correspondingly greater yield. Thus, per unit of seed weight, the growth rate was higher in plants from lighter seeds than it was in those from heavier seeds.

5. The value in water culture technique of using seeds that are nearly uniform in original weight is emphasized; such seed selection may make it possible to secure reliable results with a much smaller number of plants in each culture than would be required without selection.

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FURTHER OBSERVATIONS ON NONSYMBIOTIC GERMINATION OF ORCHID SEEDS[1]

LEWIS KNUDSON

(WITH THREE FIGURES)

In an earlier paper (5) on the nonsymbiotic germination of orchid seeds, a method was described for germinating seeds under pure culture conditions, and the successful germination of orchid seeds by the use of certain sugars was noted. The practical value of this method was indicated, and certain aspects of the problem respecting the relation of the fungus to germination were considered. It was suggested that germination is dependent upon a supply of organic substances. If the germinating orchid seed is dependent on an available supply of organic matter, then the method should be applicable to seeds of orchids other than those used in the previous experiments. The seeds successfully germinated by the use of sugar were those of *Cattleya*, *Laelia*, and *Epidendron*. No other seeds were available at that time. RAMSBOTTOM (7), reviewing this work of the writer, states that while the method may be useful in the germination of *Cattleya* and related forms, it will not prove successful for the seeds of *Odontoglossum* and related genera. He states, furthermore, that there is not much difficulty in germinating seeds of *Cattleya*, but the fact remains that under pure culture conditions *Cattleya* has not been germinated as yet in the absence of organic matter. Seeds of *Cattleya* and probably seeds of most orchids are unusual in that germination does not occur except when an outside source of carbon is available. In order to discover whether the method is applicable to other seeds, experiments were made with seeds of various genera, and that germination is possible is shown in the experiments here described. Certain other aspects of the problem are also considered.

The methods used were essentially those previously described (5). Solution B or Pfeffer's solution was used throughout, although

[1] Since the preparation of this manuscript germination of seeds of *Odontoglossum* and of *Cypripedium* has been obtained.

attention should be given to the hydrogen-ion concentration for best results. Work on this will be reported subsequently by Mr. R. S. NANZ, who is at present studying the subject. The seeds were sterilized by the use of calcium hypochlorite and the tubes and flasks were kept in the greenhouse.

CYMBIDIUM.—Seeds of a *Cymbidium* hybrid were obtained in May 1922, and twenty tubes were planted on May 8. Ten of the tubes contained solution B+2 per cent sucrose, and ten were made up with solution B alone. In all cases, 1.75 per cent agar was used. On the sugar media growth was rapid, and the mode of germination similar to that described by BERNARD (1). At the end of three months the initial leaf was formed. On the mineral nutrient solution alone, enlargement of the embryo occurred and chlorophyll was produced, but at the end of eleven months no leaf was formed. The germinated seedlings were transplanted September 1 to liter flasks of Erlenmeyer form, each of which contained 300 cc. of solution B+2 per cent sucrose. Growth of these seedlings continued rapidly, and despite the fact that over 100 seedlings were planted in each flask, at the end of eleven months the seedlings averaged 5 cm. in length, having three or four leaves and well developed roots (fig. 1).

ODONTOGLOSSUM.—On the same date a similar experiment was started with seeds of *Odontoglossum Rossii*×*Odontioda* hybrid. These and the seeds of *Cymbidium* were obtained from Mr. A. C. BURRAGE, of Boston, Massachusetts. The results were the same as with *Cymbidium*, except that after transplanting, the seedlings apparently went into a resting condition, growth being resumed near the middle of January. At present the seedlings average 2 cm. in height, with roots developing.

PHALAENOPSIS SCHILLERIANA.—On November 10, 1921, seeds were sown in tubes containing solution B+1 per cent glucose or sucrose. These germinated, and by the end of four months the initial leaf was appearing. Unfortunately, they were transplanted to small flasks in which the agar soon dried up. These seedlings were transplanted again in December 1922. Growth again took place, so that one and two leaves are now formed, and roots four or five mm. in length are now present. Undoubtedly more satis-

factory and rapid growth would have been obtained had proper attention been given to the hydrogen-ion concentration.

OPHRYS.—Seeds of four species of *Ophrys* (*O. fusca*, *O. speculum*, *O. apifera*, and *O. fragrans*) were obtained from Spain, and planted on December 26, 1922. On April 1, 1923, the seeds were germinated. The protocorm at this time was a large spherule about 1.5 mm. in diameter, with many radiating absorbing hairs, some of them 1 cm. long. The initial leaf was just appearing. The protocorm was colorless, while the initial leaf was a deep green.

FIG. 1.—Seedlings of *Cymbidium;* 4/5 natural size

DENDROBIUM.—Various experiments have been made with seeds of *Dendrobium nobilis*. No difficulty has been noted in germinating these seeds. After germination in tubes the seedlings were transplanted to flasks, and in from six to seven months the seedlings had four and five leaves, and a small pseudo bulb was formed.

Thus far I have failed only with the seeds of two genera, and I am not certain that the method is at fault, but suspect that the seeds were not viable. Mr. SANDERS of Belgium brought with him from that country a pod of *Odontoglossum* which he gave to me after several

weeks of traveling. The pod when received was blackish green, but firm. The seed when examined microscopically appeared immature, although I am not certain that this was the actual case, not being very familiar with seeds of this genus. This seed failed to germinate. In another experiment, seeds of *Cypripedium venustum* were planted, and these also failed to germinate. The age of the seeds was not known, so that failure may have been due to loss in viability.

To sum up this phase of the work, it may be stated that germination has been noted for seeds of *Cattleya*, *Laelia*, *Epidendron*, *Cymbidium*, *Phalaenopsis*, *Dendrobium*, *Ophrys*, and a hybrid of *Odontoglossum*×*Odontioda*. In all cases the germination has been practically 100 per cent.

VIABILITY OF ORCHID SEEDS.—Through the courtesy of LAGER and HURRELL of Summit, New Jersey, seeds of *Cattleya* and related forms and hybrids were obtained for experiments on viability. These were planted on November 6, 1922, and observations recorded on March 1, 1923. Previous to being received, these seeds were kept in a rather warm dry office in paper packages. Five lots of seeds one year old or less germinated readily. Two lots of seeds about fifteen months old did not germinate. Some of the more interesting observations were made with old seeds. Thus seeds of *Cattleya Trianae* which had been stored for three years showed 40 per cent germination. Seeds of *Cattleya Dowiana*×*C. Faleia* which had been stored four years germinated to the extent of 1 per cent. This percentage of germination was obtained with other seeds of this age. Various other lots of seeds three years of age showed 1–10 per cent germination. With seeds of *Cattleya* there is apparently some variability in the duration of viability. No difficulty has been experienced in germinating fresh seeds, but with seeds a year or more old the results are uncertain. It would seem from this that seeds should be planted as soon as possible after collection.

NECESSITY OF ORGANIC MATTER FOR GERMINATION.—In certain experiments, BERNARD found that germination occurred when he used a concentrated solution of salep. This would contain sugars and other organic substances. He ascribed the beneficial effect

of the increased concentration to a physical chemical effect, and compared the action with that of high concentrations in changing the form of algae. Recently, RAMSBOTTOM has implied that the effect of the fungus or of high concentration of salep is comparable with the activating influence of various salts and chemical reagents on inducing the parthenogenetic development of certain eggs. That this is not the true explanation in the case of sugars is evident from the various experiments. Whenever the seeds are sown on media containing the appropriate sugar, that sugar is absorbed and stored as starch in the embryo, but not all sugars are utilized. If lactose is used at the same molecular concentration as glucose, fructose, or sucrose, the seed behaves as though no sugar were present. Lactose is not generally utilized by green plants as a food.

That the sugar is used as a food is suggested again by the following experiment. On January 30, 1922, seeds of a *Cattleya* hybrid were sown in tubes on solution B+2 per cent glucose. These cultures were kept in a dark incubator with the temperature maintained at 25° C. On May 3, the seedlings were well advanced and bearing one or two leaves (fig. 2). The seedlings were very much etiolated, but when transplanted to flasks they quickly became green, and are now making a normal growth. The first observations were made on these seedlings on April 8, 1922. At that time the embryos were gorged with starch, and averaged close to 0.5 mm. in width. The same results reported for glucose were also obtained when mannose was used. Without sugar no germination occurred. BURGEFF (4) attempted to germinate seeds of *Cattleya* in the dark, supplying sucrose in the nutrient medium, but he reported that germination was not possible under these conditions. The concentration of sugar was too low and the time of incubation probably not long enough.

In all of these experiments attempts were made to germinate the seeds on nutrient media containing only the essential salts, but lacking in sugar. In all cases germination failed to occur. With seeds of *Cattleya*, no germination was noted even after two years on such media, despite the fact that the embryos were transplanted several times because of the tendency of the media to evaporate. The embryos became green after the first week or so, and gradually

increased in size, so that at the end of three months they were 0.2–0.3 mm. in width, and at the end of a year some were found to be 0.5 mm. Beyond this, no further development was noted.

What is the cause of this failure to germinate? Chlorophyll is apparently present; water is readily absorbed; delicate absorbing hairs may be formed; all of which indicate complete permeability to salts and gases. It appears likely that the chlorophyll does not function because of the lack of some internal factor which is essential for the photosynthetic process. This would be in line with the recent observations made by BRIGGS (**2, 3**) and others that the initiation of photosynthetic activity in young leaves is dependent on some internal factor. The photosynthetic activity lags behind chlorophyll development. The fact as reported by BRIGGS that the cotyledons of *Helianthus* after chlorophyll development do not show this delay in the photosynthetic process suggests that food materials are involved. The facts that the orchid seed has practically no reserve food, and that if germination proceeds to a certain stage the embryo becomes independent of any outside food, make the preceding hypothesis more plausible.

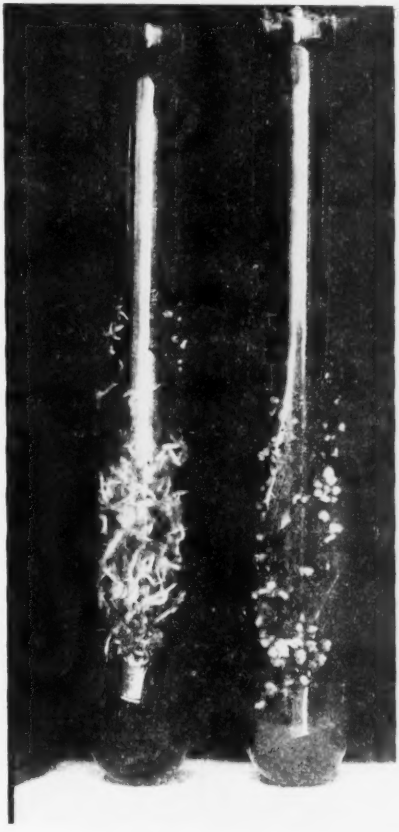

FIG. 2.—Seedlings of *Cattleya* germinated in dark; smaller seedlings on 4 per cent and larger ones on 2 per cent glucose; 4/5 natural size.

It is made apparent from a number of experiments that once germination has proceeded to a certain point, the seedlings become independent of sugar supplied by the medium and independent of any fungus. Seeds of *Cattleya* were sown on solution B + 2 per cent sugar in the usual way. When the leaf point was just appearing, that is, when the embryos were about 0.7–0.8 mm. in width, they were transplanted to flasks containing solution B or Pfeffer's solution. No sugar was supplied. While slower than in those cultures with sugar, growth was continuous, and well developed plants were obtained (fig. 3). These results have been noted

FIG. 3.—Two-year old *Cattleya* and *Laelia Cattleya* seedlings on full nutrient solutions without sugar; 2/5 natural size.

not only in these two cultures, but in many other cultures of like character.

SUMMARY.—These experiments lend further support to the hypothesis that the germination of orchid seeds is dependent on an outside source of organic matter. The effect of sugar on germination is that of a food, but when germination has proceeded to the stage when the leaf is just appearing, then subsequent growth may occur in the absence of any available organic matter in the nutrient medium. This leads to the idea that while the embryos possess chlorophyll, the photosynthetic process does not occur, probably owing to the lack of some internal factor. While these experiments

do not disprove the necessity of the fungus for germination under certain conditions, they do emphasize again the nutritional aspects of the problem. At the present time experiments are in progress on the so-called symbiotic germination, and the results thus far obtained lend support to the ideas expressed in my previous paper in respect to the function of the fungus.

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EFFECT OF TEMPERATURE AND LIGHT ON CATALASE CONTENT OF SPIROGYRA

W. E. Burge and E. L. Burge

(with two figures)

As a result of the work of Lavoisier (6) on animals, and of Priestly (7), Ingen-Housz (5), Senebier (8), and De Saussure (4) on plants, it is now known that both animals and plants in their respiratory processes take up oxygen from the air and give off carbon dioxide. There are many ways of increasing, as well as decreasing this oxygen absorption and carbon dioxide elimination. Exercise, food, cold weather, cold baths are some of the ways by which the gaseous exchange in animals may be increased, while it is decreased during rest, starvation, and anesthesia. The respiratory metabolism in plants may also be increased or decreased in various ways, one of the most effective being by varying the temperature. Light produces only a slight increase in the respiratory metabolism of plants, although it is essential for photosynthesis (2).

During the past several years we have found that whatever increases the respiratory metabolism in animals, whether it be food, exercise, cold baths, or cold weather, invariably produces an increase in catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide; and whatever decreases the respiratory metabolism, as rest, starvation, anesthetics, decreases this enzyme. The object of the present investigation was to determine whether an increase or decrease in oxidation in a plant is accompanied by a corresponding increase or decrease in catalase similar to that found to be the case in animals. It should be mentioned in this connection that evidence bearing on this point has already been furnished by Appleman (1) and Crocker and Harrington (3). Appleman found in the greening and sprouting of potatoes an increase in catalase corresponding with the increase in respiration. Crocker and Harrington found a similar relation between catalase and respiration in the germination of seeds.

The plant used in this investigation was *Spirogyra*, and the respiratory metabolism was decreased by lowering the temperature and increased by raising it. The effect of light was also studied. We were fortunate in having an almost inexhaustible supply of a practically pure culture of *Spirogyra porticalis*[1] in a nearby lake. The lake was frozen almost continuously during the months of January and February, when most of the experiments reported in this paper were carried out.

The catalase determinations were made as follows. The desired amount of *Spirogyra* was placed in a cloth and the excess water removed by squeezing. This material was then ground through a small hashing machine twice, and 1.25 gm. of it added to 15 cc. of neutral hydrogen peroxide at 18° C. in a bottle. The amount of oxygen liberated in twenty minutes was taken as measure of the catalase content. The bottles were shaken at a slow rate in a shaking machine during the determinations.

On several successive days the ice on the lake was broken, *Spirogyra* collected, and catalase determinations made. It was found that 1.25 gm. of the macerated *Spirogyra* liberated on the average 27 cc. of oxygen from neutral hydrogen peroxide in twenty minutes, the greatest amount of oxygen liberated being 29 cc. and least 25 cc. A quantity of *Spirogyra* was removed in the ice-cold water to the warm laboratory (22° C.) and catalase determinations made immediately, after four, and after twelve days. The material which was used immediately liberated 29 cc. of oxygen from hydrogen peroxide in twenty minutes; that kept in the warm laboratory for four days, 52 cc., and that kept twelve days, 62 cc. It may be seen that the removal of *Spirogyra* from the cold water of the lake where it had been during the winter to the warm laboratory resulted in more than doubling the catalase content in fourteen days. This is in keeping with the fact that the respiratory metabolism of plants is increased by raising the temperature.

The object of the following experiments was to determine the effect of different temperatures and of artificial light on the catalase content of *Spirogyra*. A large quantity of *Spirogyra* was collected and brought to the laboratory in ice-cold water. It was found that

[1] We are indebted to Dr. STELLA HAGUE for identifying this species of *Spirogyra*.

1.25 gm. of this material liberated 31 cc. of oxygen from hydrogen peroxide in twenty minutes. The large batch of *Spirogyra* was divided into six portions, each of which was placed in 10 l. of lake water in a container 40 cm. in diameter. Three of the portions were placed in the dark at 0°, 18°, and 30° C., while the remaining three were kept at the same temperatures and each exposed to a 200 watt light with a frosted bulb at a distance of 1 m. Catalase determinations were made at certain intervals. The results are given in fig. 1.

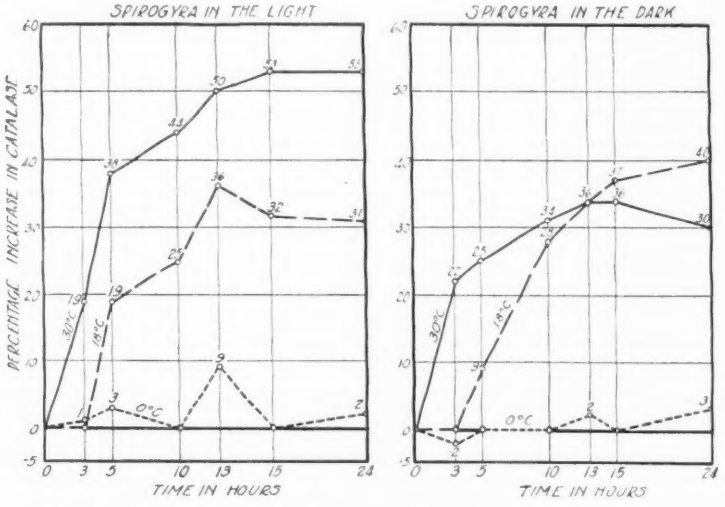

FIG. 1.—Curves showing that rise in temperature increases catalase of *Spirogyra* in light as well as in dark, and that increase is greater in light than dark.

There was no change in the catalase content of the *Spirogyra* kept either in the light or in the dark at 0°, while there was an increase in that kept at 18° and 30° C., both in the light and in the dark. The increase in catalase of the *Spirogyra* kept in the light at 30° was greater than that kept in the dark at the same temperature. This is in keeping with the fact that light, independent of the heat effect, produces an increase in the respiratory metabolism of plants. Two other similar experiments were carried out with essentially the same results.

In fig. 2 is shown the effect on the catalase of keeping *Spirogyra* under ordinary conditions of day and night at 18° and 0° C. The *Spirogyra* that was kept at 18° was placed in the laboratory before a window where the sun could shine on it most of the day, while that kept at 0° was on the roof. No change was produced in the catalase content of the *Spirogyra* kept at 0°, while there was an increase in that at 18° C. After seventy-six hours, a part of the material that was being kept in the laboratory at 18° was lowered to a temperature of 0° C., and the effect was to decrease the catalase greatly.

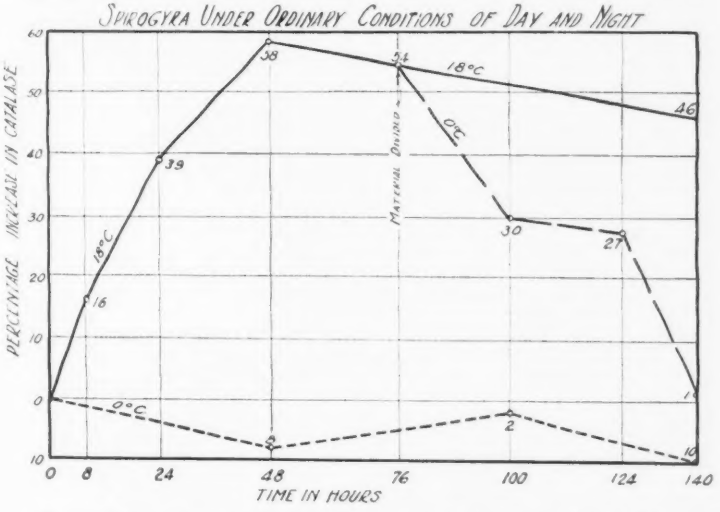

FIG. 2.—Curves showing that rise in temperature increases catalase of *Spirogyra* and a fall decreases it under ordinary conditions of day and night.

Summary

1. A fall in temperature produces a decrease in the catalase of *Spirogyra*, and a rise in temperature an increase, in keeping with the fact that a fall in temperature decreases and a rise in temperature increases the respiratory metabolism of plants.

2. Light also produces an increase in the catalase of *Spirogyra*, less extensive, however, than that brought about by a rise in temperature, in keeping with the fact that light is less effective than a rise in temperature in increasing the metabolism of plants.

3. Whatever increases or decreases the respiratory metabolism in animals produces a corresponding increase or decrease in catalase. From the work here reported and the work of others on the subject, it would seem that it is probable that this same relationship exists between catalase and the respiratory metabolism in plants. Hence the suggestion is made that catalase may be the enzyme in both plants and animals which is principally responsible for the respiratory metabolism.

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DEVELOPMENT OF SEED OF LINARIA VULGARIS

MELVILLE T. COOK

(WITH PLATE XVI)

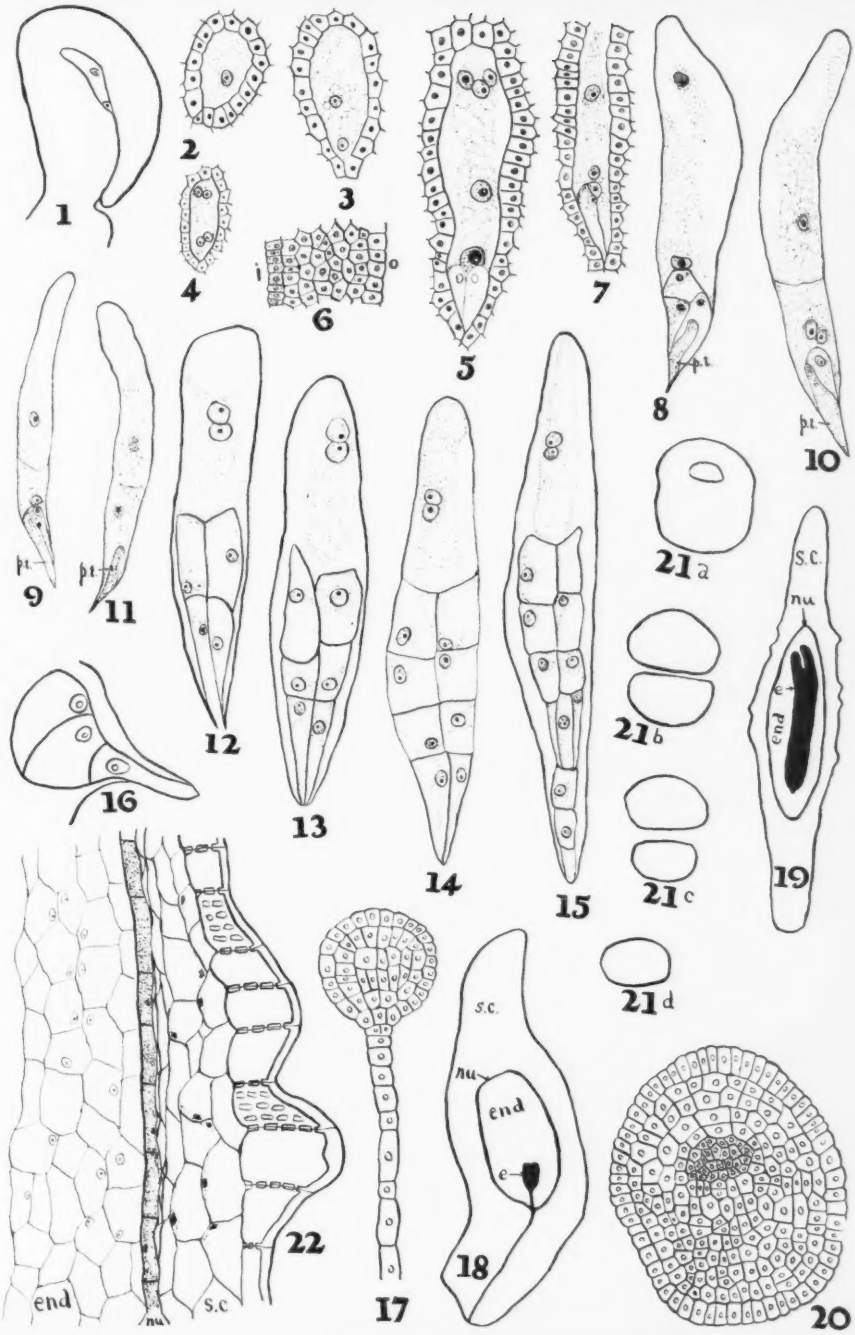
Linaria vulgaris is an introduced plant belonging to the Scrophulariaceae. It bears a large number of very small seeds, but probably reproduces most generally by means of vegetative organs. The embryo sac is located deep within the ovule, but the pathway of the pollen tube is well defined (figs. 1, 18). The embryo sac develops in the normal manner (figs. 2–5), and is bounded by well-defined, more or less cubical cells, which are richer in protoplasm than the other cells of the nucleus (figs. 2–7). The synergids and antipodals disintegrate very quickly. The polar nuclei unite in the normal manner, but triple fusion was not observed.

The pollen tube passes through a very small canal, enlarges, and becomes very prominent after entering the embryo sac (figs. 1, 8–11). Fertilization was not observed, but probably occurs in the normal manner. The proembryo divides by two transverse walls, the two upper cells enlarging and developing into a spherical embryo, and the lower cell developing into a long suspensor consisting of a single row of cells (figs. 16, 17). This spherical embryo gives rise to the cotyledons and other structures (fig. 17), and at this period bears a striking resemblance to the corresponding stage of the embryo of *Capsella bursa-pastoris*. The development of the cotyledons is unequal, one starting somewhat in advance of the other and developing into a slightly larger organ (figs. 19–21). A cross-section of the embryo just below the cotyledons at this stage shows the fibrovascular bundle of one developing a little in advance of the other (fig. 20), and successive sections farther up show that one cotyledon is always slightly larger than the other (fig. 21*a–d*).

The endosperm is much more interesting, and may develop although the embryo does not. In fact, the majority of seeds are without embryos. In some cases all the seeds in an ovary may be without embryos. The development of the endosperm, however, is the same regardless of the presence or absence of the embryo. In

some cases the pollen tube enters a sac in which no embryo is formed (fig. 11), but in most cases it is doubtful whether the ovule is penetrated by the pollen tube. The endosperm is of the cellular type, and the walls are well defined. The first division is transverse (figs. 9–11). After this, the development of the endosperm is from the cell next to the micropyle, which undergoes repeated transverse and longitudinal divisions (figs. 10, 12–15). The cells of the endosperm at this stage tend to shrink in most cases from the action of the killing fluid, and give very much the appearance of an embryo in those ovules in which there is no embryo. In fact, the resemblance is so striking that the writer was deceived for some time. The nucleus in the antipodal end of the sac divides, but no wall is formed. The two nuclei lying side by side have very much the appearance of two polar nuclei, and help in the deception (figs. 12–15). The writer was unable to determine their final fate, but it is doubtful whether they serve any further function. A division of the sac into two chambers and the development of the endosperm in the micropylar end of the sac have been described for many species among both monocotyledonous and dicotyledonous plants, including the Scrophulariaceae. The behavior of the endosperm nucleus in the antipodal end of the sac varies in different species; in some there is little or no change, in others there is an enlargement, in others a division into two or three nuclei, and in others a disintegration. The endosperm of *L. vulgaris* finally develops into a compact mass of small cells with thin but well defined walls. These cells are rich in protoplasm until or just before the starting of the cotyledons (fig. 18), when the protoplasm and nuclei begin to disappear and starch is formed in great abundance. The nucellus disappears very rapidly, and at the time of the beginning of the cotyledons it is reduced to a single layer of cells which persists in the mature seed (figs. 18, 19, 22).

The mature seed consists of a very long embryo with short unequal cotyledons (fig. 19), imbedded in a mass of endosperm consisting of large cells filled with starch (figs. 19, 22), surrounded by a single layer of nucellar cells which are rich in protoplasm; all of which is surrounded by a seed coat. This seed coat consists of large thin-walled cells containing practically no food or protoplasm, and an outer layer of large thick-walled pitted cells.



COOK on LINARIA

This work was done at the New York Botanical Garden while the writer was on leave of absence from Rutgers College.

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EXPLANATION OF PLATE XVI

FIG. 1.—Ovule, showing embryo sac and pollen tube canal through nucellus.

FIG. 2.—One-celled embryo sac.

FIG. 3.—Two-celled embryo sac.

FIG. 4.—Four-celled embryo sac.

FIG. 5.—Eight-celled embryo sac.

FIG. 6.—Nucellus at time of completion of embryo sac (*i*, inner wall; *o*, outer wall).

FIG. 7.—Embryo sac just before fertilization; no union of polar nuclei.

FIG. 8.—Embryo sac just after fertilization; endosperm nucleus has undergone first division; also showing pollen tube (*pt*).

FIG. 9.—Embryo sac with one-celled embryo, pollen tube, first division of endosperm nucleus and wall.

FIG. 10—Same as fig. 9, but also showing division of endosperm nucleus next to micropyle, but with second wall as yet unformed.

FIG. 11.—First division of endosperm nucleus; no embryo; pollen tube present.

FIG. 12.—Endosperm but no embryo; note two nuclei formed from division of endosperm nucleus in antipodal end of embryo sac.

FIG. 13.—Same as fig. 12 but slightly older.

FIG. 14.—Same as fig. 13 but slightly older.

FIG. 15.—Same as fig. 14 but slightly older.

FIG. 16.—Three-celled embryo.

FIG. 17.—Spherical embryo with long suspensor.

FIG. 18.—Diagram of seed, showing young embryo (*e*), endosperm (*end*), nucellus (*nu*), and seed coats (*sc*).

FIG. 19.—Same as fig. 18, but showing older embryo.

FIG. 20.—Cross-section of embryo just below cotyledons; shaded part indicates fibrovascular bundle in older cotyledon.

FIG. 21*a*, *b*, *c*, *d*.—Four successive cross-sections of embryo as in fig. 20; *a*, same as fig. 20; others in cotyledon region showing differences in two cotyledons.

FIG. 22.—Section of fig. 20, showing endospern, nucellus, and seed coat.

BRIEFER ARTICLES

CHARLES FREDERICK MILLSPAUGH

(WITH PORTRAIT)

CHARLES FREDERICK MILLSPAUGH was born in Ithaca, New York, in 1854, and died at Chicago, Illinois, in 1923. As a child he displayed a strong interest in outdoor sports and in objects of natural history. It is related that in early boyhood he attracted the notice of the great LOUIS AGASSIZ when the latter was fishing near Ithaca, this chance meeting soon ripening into a lasting friendship between the two. A nephew on his maternal side of EZRA CORNELL, the founder of Cornell University, he spent the years 1872–75 as a student at Cornell. A few years later he went to the New York Homeopathic Medical College, where he was granted the degree of Doctor of Medicine in 1881.

For nine years following the receipt of his doctorate he practiced medicine in Binghamton, New York. Early during this period, however, he appears to have been lured by the charm of plant study. As with TORREY, GRAY, ENGELMANN, and so many other pioneers of American botany, his work along medical lines rapidly came to be eclipsed by an absorbing interest in plants. In 1887 he published his extensive work on *American Medicinal Plants*, one of the monumental American works in its line. This work was issued in ten volumes, with a total of one hundred-eighty full page colored plates delineated and painted by himself. Only those who have essayed to portray plants on such a comprehensive scale can appreciate the immense amount of labor that these plates represent. Their beauty and fidelity to nature are a credit, not only to the ability and accuracy of their artist, but also to the able instruction received from his father, JOHN HILL MILLSPAUGH, to whom he publicly expressed his indebtedness for whatever he might possess "of art in coloring and drawing."

In 1891–92 he was Professor of Botany at the University of West Virginia, forming an intimacy with the flora of that state which found expression in various papers, the most extended being his *Preliminary Catalogue of the Flora of West Virginia* (1891) and his *Flora of West Virginia* (Edit. I, 1896; Edit. II, 1913). In 1894 he was made Curator

From photograph by Eve Watson Shutze

CHARLES FREDERICK MILLSPAUGH

of Botany at the Field Museum of Natural History, Chicago, where he remained until his death. In rapid succession he produced various works of a taxonomic nature. These dealt chiefly with the North American Euphorbiaceae, the floras of Yucatan, the Bahama Islands, St. Croix and others of the West Indies, and Santa Catalina Island. Nor did he refrain from activities in the field. At various times he collected in different parts of the United States from Massachusetts to California, in the West Indies, Mexico, Brazil, and elsewhere. His plant labels at the Field Museum show that he made no less than four trips to Yucatan. In 1911–12 he made a journey around the world. Experienced at collecting and expert as a photographer, he returned with a vast number of specimens and photographs of scientific interest.

During his long and active botanical career many honors were bestowed upon him. He was Professorial Lecturer in Botany at the University of Chicago in 1895, and later was Associate Professor of Economic Botany at the same institution. In 1896 he was made Professor of Medical Botany at the Chicago Homeopathic Medical College. He was a Fellow of the American Association for the Advancement of Science, of the Faculdad de Medicina, Mexico, and the Faculdad de Medicina, Brazil; a member of the Torrey Botanical Club, the Explorers' Club of New York, Sigma Xi, and many other societies. In recent years he was a dominant figure in the American Wild Flower Preservation Society.

Toward the close of 1919, in an effort to recuperate his physical strength after a serious operation, he went to the Island of Santa Catalina, California. There he collected many specimens of plants, took numerous field notes and photographs, and laid the foundation for his *Flora of Santa Catalina Island*, which he published in collaboration with L. W. NUTTALL in January, 1923. This was his last published work.

The great herbarium and plant exhibits organized at the Field Museum under his personal direction will long pay eloquent tribute to his energy and enthusiasm. Probably nowhere else among the larger herbaria of the world are the plants so thoroughly indexed as to data of collection. During the past decade there have been made and installed at the Field Museum many beautiful and justly famous models of representative plants, which have proved at once the marvel and delight of visiting botanists from all quarters of the globe. He labored indefatigably to assemble at the Field Museum a great collection of valuable materials that some day should make Chicago a foremost center of taxonomic research.

To the younger generation of American botanists Dr. MILLSPAUGH was known mainly by reputation, for, busied with the many administrative tasks incident to his work, he often had to forego attendance upon conventions of fellow scientists. A full six feet in stature, of erect carriage and decisive manner, he possessed a strong and positive personality not soon to be forgotten by those who knew him. Accurately to appraise his various qualities and powers as a botanist would be well-nigh impossible, nor would the writer, biased with the warm friendship that comes from an acquaintance and close personal contact during the past twelve years, feel equal to such a task. True it is, however, that Dr. MILLSPAUGH's death marks the departure of an able taxonomic worker and one of America's most brilliant organizers of museum displays. —E. E. SHERFF.

USE OF NEGATIVE PRINTS FOR COMPARING MICROSCOPIC STRUCTURES

(WITH ONE FIGURE)

Recently the little used method of making photographs directly on sensitive paper has been found very satisfactory at the Forest Products Laboratory for obtaining enlarged photographs of wood structure for study.[1] Such photomicrographs made from very thin sections of wood, mounted in the usual manner, furnish permanent, easily available records of material investigated. They permit study and direct comparison of the minute structure at any desired magnification without the aid of a microscope. The photographs are negative of course, but this does not affect their usefulness; in fact, it has some advantages.

Permanent or temporary slide mounts may be used. In this case, permanent mounts of thin sections of wood, 10–20 $\mu\mu$ in thickness were photographed. These sections were colored with selective stains, using contrasting cardinal colors, such as reds and blues. As wood elements have an affinity for some colors with varying degrees of absorption, differential coloring was produced which showed as white, black, or intermediate grays in the photomicrographs.

In connection with a study on the occurrence of brashness in wood, it was found necessary to measure the areas occupied by the wood fibers on cross-sections of the wood in over one hundred microscopic sections

[1] Assistance in preparing photomicrographs was given by Dr. M. E. DIEMER, chemist and photographer at the Forest Products Laboratory.

of the wood of white oak. In the photomicrographs the fiber areas (white in the photographs) were clearly differentiated. An ordinary

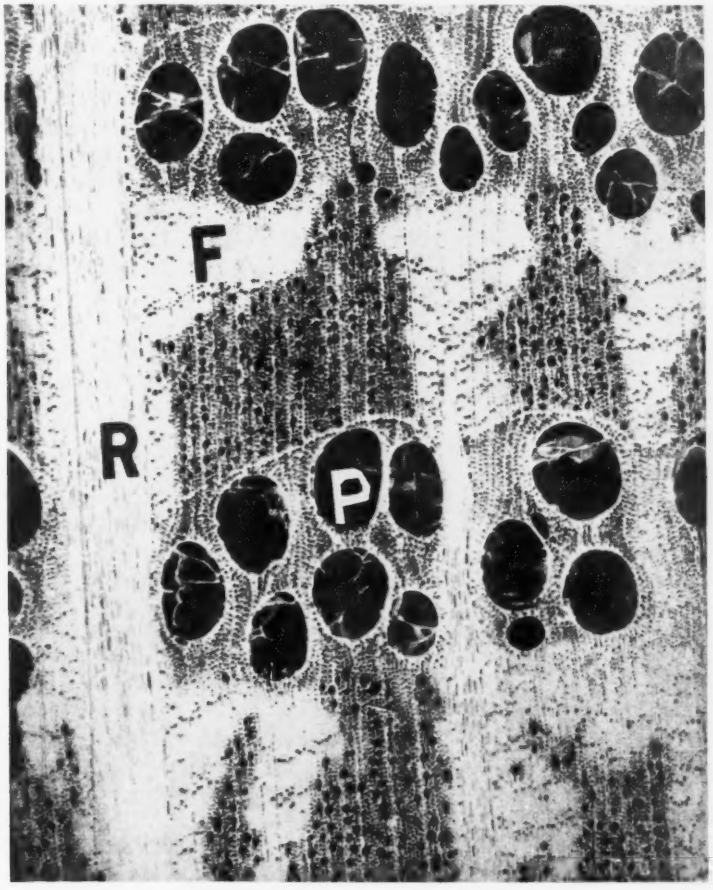

FIG. 1.—Negative print of cross-section through portion of two growth rings of white oak; *P*, pore; *R*, ray; *F*, wood fiber area.

Dietzgen polar planimeter was used to measure these areas. Simple calculations gave the percentage of wood fiber area for each section.

Ordinarily, exposures are made on commercial dry plates or films from which later positives prints are obtained. The procedure adopted here dispenses with this step. It is inexpensive, a great time saver, and comparatively simple. The photomicrographic equipment used consisted of a carbon arc lamp on a 220-volt circuit, its housing, a microscope of the large-barrel type, a camera bellows, and focusing screen of ground glass. Only an objective is necessary with the microscope for lower magnifications. The equipment must be rigid and all parts in proper relative adjustment to secure sharp photographs. A long bellows is best, as it permits working wholly within the light circle and allows large plates to be covered.

Essentially, the process involves the making of negatives on ordinary photographic printing paper placed behind a clear glass in the plate holder. The exposure varies from 25 to 50 seconds, depending on intensity of light and thickness of wood sections. These negative prints are treated in the dark room in the same manner as paper which has been exposed behind the ordinary negative. For most work glossy prints are preferable.

Approximately the same conditions of light and shadow in the wood as are apparent to the eye are reproduced; that is, the pores are dark and the rays are light or white. Only the wood fiber areas are negative; they appear lighter than the surrounding tissues in the photograph, whereas on the end grain of a piece of oak they appear darker. The interchange of black and white by the use of negative prints is an advantage over the ordinary print in demonstration work, for the reason that the picture more nearly simulates actual conditions on viewing the end grain of the wood itself.—REUBEN W. SMITH, *Forest Products Laboratory, Madison, Wis.*

CURRENT LITERATURE

BOOK REVIEWS

Researches on fungi

The observations and experimental studies reported in BULLER's *Researches on fungi*, published in 1909, have been continued, and so amplified that the original volume is now to be only the first of four. The second volume[1] has recently appeared, and the remaining two are said to be in an advanced stage of preparation for the press.

The study of the problem of spore discharge in the Hymenomycetes is continued. It is shown that each spore has a slight projection, the hilum, just above the point of attachment to its sterigma and pointing toward the central axis of the basidium. From the hilum, just before spore discharge, a tiny drop of fluid is extruded. This drop is always carried away with the spore. What changes occur in the spore and in the basidium at this time, and exactly how the violent spore discharge is brought about, remain to be discovered. The violent discharge itself is shown to occur not only in agarics, but in species belonging to the related families Polyporaceae, Clavariaceae, Hydnaceae, and Thelephoraceae, and also to the gelatinous families Tremellaceae, Dacryomycetaceae, and Auriculariaceae, and to the rusts, and is probably characteristic of all basidia borne in an exposed hymenium. Detailed discussion of the discharge in some of these groups is reserved for the future volumes. It is pointed out that the tremellaceous groups, in spite of their wide separation phylogenetically from the Hymeniales, have not only developed a similar mechanism for spore discharge, but have evolved pileate forms with spines, pores, and even gills, thus furnishing a striking illustration of phyletic convergence.

The major part of the volume is devoted to a careful analysis of the hymenium in agarics. Two distinct types are recognized: the non-*Coprinus* or Aequi-hymeniiferous type, and the *Coprinus* or Inaequi-hymeniiferous type. In the former type, which includes all genera of agarics except *Coprinus*, the gills are wedge-shaped in section and are positively geotropic; hence in a normally oriented hymenium, every part is directed toward the center of the earth. Spores are liberated from every part of the gills throughout the period of spore discharge, which may continue for several days, and each spore, on its discharge, is shot perpendicularly into the interlamellar space for a distance of 0.1–0.2 mm.,

[1] BULLER, A. H. R., Researches on fungi. Vol. II. Further investigations upon the production and liberation of spores in Hymenomycetes. 8vo. pp. xii+492. *figs.* 157. London: Longmans, Green, & Co. 1922.

after which its trajectory changes abruptly and it drops at a uniform rate to the outer air, where it becomes subject to air currents.

In the *Coprinus* type of fruit body the gills are relatively thin, with sides almost or wholly parallel, and do not respond to the stimulus of gravity; hence, under natural conditions, part of the hymenium may be directed upward. The basidia are not matured simultaneously on all parts of the gills, but from below upward, so that all the basidia on a given level ripen their spores before the spores on the basidia above them have matured; hence the spores are discharged in regular succession, beginning at the bottom. As the zone from which the spores have been discharged advances from below upward on each gill, it is immediately dissolved by a process of autodigestion. The pileus flesh is thin and is also subjected to autodigestion after the spores have been discharged.

Each of these types is subdivided into several subtypes, and nearly half of the volume is devoted to a detailed consideration of the organization of the hymenium in *Panaeolus campanulatus*, *Anellaria separata*, and *Psalliota campestris*, all representing the *Panaeolus* subtype of the Aequi-hymeniiferae.

The author points out that no really satisfactory drawing of the hymenium, showing accurately the space relations of the hymenial elements one to another, has heretofore been published; that the behavior of a basidium after its spores are shed has never been followed; and that the distinction between basidia and paraphyses, and the rôle played by the latter elements, have been vague and uncertain. These deficiencies he supplies, publishing numerous beautiful illustrations and drawings, of which special reference should be made to figs. 96 and 147, illustrating the hymenium of *Panaeolus campanulatus* and of *Psalliota campestris* respectively. He shows that each basidium bears but one set of spores and then collapses, and that the paraphyses may, with ordinary care, be distinguished satisfactorily from the developing basidia. It is shown that as the basidia of successive generations collapse after discharging their spores, the paraphyses enlarge and become more and more prominent, thus keeping the hymenium turgid until the period of spore discharge, which may last for a week or more, is brought to its conclusion.

Other matters treated include xerophytism in tremelloid and non-tremelloid fruit bodies, variation in *Coprinus* and *Marasmius*, and squirrels and slugs as mycophagists. In keeping with its character, the book is notably free from typographical errors, and its one hundred fifty-seven illustrations are admirably selected, beautifully executed, and always pertinent.—G. W. MARTIN.

Saprolegniaceae

The results of many years of study of the water molds are brought together in a monograph by Coker[2] giving an account of all known species, with figures of all forms that he has observed in the living state. These illustrations cover 63 full page plates of excellent ink drawings, and constitute a very important part

[2] COKER, W. C., The Saprolegniaceae, with notes on other water molds. University of North Carolina Press. pp. 201. *pls.* 63. 1923.

of the contribution, especially since there are shown for each species usually a number of stages in the life history. This is the first extensive treatment of this group of fungi as a whole since HUMPHREY'S monograph of 1893 and FISHER'S account of 1892 in RABENHORST'S *Flora*.

Thirty years have added much of cytological and physiological interest, which shows itself in the character of the taxonomic treatment. For example, the description of *Saprolegnia ferax* with its synonymy, distribution, and references to other illustrations covers less than one and a half pages, but then follow five pages chiefly given to a discussion of the behavior of this species when grown under various cultural conditions. As regards taxonomic practice, it would appear that some species of water molds can with certainty be identified only after cultural tests.

Throughout the monograph there is frequent reference to the behavior of species when grown in various media. This is interesting reading, but one wishes that some general conclusions had been formulated and brought together on the effects of physiological conditions, since these seem to play so important a part in the expression of the plant's morphology. There are also numerous references to points of cytological interest in connection with particular species, but no general summary covering this matter for the group as a whole. The subject of chondriosomes receives considerable attention. The seasonal habits of a number of species are described and compared from extensive observations based chiefly on collections from the vicinity of Chapel Hall, North Carolina. In this more moderate climate there is no closed season, and water molds may be found in open water throughout the winter. This contrasts with the findings of PETERSON from studies in Denmark, where the colder winters generally confine the species to the months of spring, summer, and winter.

In all some 60 species are described from the author's personal studies, and quotations are given from descriptions of about 30 other species not known to America. Several pages and two plates, devoted to fungus parasites upon the water molds, will be welcomed by students of the group. The bibliography lists 234 titles.—B. M. DAVIS.

Heredity and chromosomes

A good translation into German makes available to a much wider constituency STOMPS'[3] recent work in Dutch on heredity and cytology. This is a readily intelligible account, and while no new ground is broken, the collection of material and the mode of presentation make it a useful book.

It is divided into three parts: the first, a treatise on the cell, cell division, and the reduction division; the second, entitled heredity, but really a short account of DE VRIES' intracellular pangenesis theory, with evidence regarding the functions of the nucleus; and the third, a more lengthy exposition of the relation of chromosomes and heredity. As regards cell division, the author

[3] STOMPS, THEO. J., Erblichkeit und Chromosomen, pp. viii+158. *figs. 24*. Jena: Gustav Fischer. 1923.

claims that, owing to the accompanying vacuolization and the increase and decrease in vacuole size as regards nuclei, chromosomes, and protoplasm, mitosis may be regarded as an osmotic phenomenon. He considers that the pulling fibers may be permanently united between the chromosomes and the general protoplasm of the cell, although not visible in the resting stage. He holds with GATES that in the reduction division both telosynapsis and parasynapsis are possible. The material of the third part is presented chiefly in the form of an account of the classic researches of BOVERI and HERBST on sea urchins, and of MORGAN and BRIDGES on *Drosophila*. He considers that MORGAN's work will convince all reasonable people that the physical basis of Mendelian properties in *Drosophila* is in the chromosomes, and that what is true of this species must be the case with all others. Mendelism itself receives scant attention and that near the close of the book. This treatment would appear to be both a defect of proportion and a cause of difficulty to readers unfamiliar with the subject.

In general, however, the book is well written, and the historical method of presentation, which has been adopted throughout, has been cleverly utilized. It should prove very useful to those who require a simple, accurate, and up-to-date account of the chromosome theory of heredity.—R. O. EARL.

Plant biology

In a text book intended primarily for medical students and others who do not intend to continue the study of botany, TANSLEY[4] has arranged carefully selected material in a decidedly attractive manner.

After a brief introductory chapter, the author discusses organic substances and their chemical characters, and then the physical nature of organic substances, including the differences between crystaloids and colloids, gel formation, and the processes of diffusion and osmosis. The remainder of the book makes a survey of plant forms from *Protococcus* through the customary groups to the flowering plants. Type plants are taken with a view to using them as a basis for the discussion of biological principles. The student should have a fair understanding of such principles and the essential life processes of plants rather than detailed knowledge of plant structures and plant classification at the close of the book, which seems a very desirable result. The book is clearly and interestingly written, and seems thoroughly modern in the subject matter presented and the information imparted. Practical exercises are appended to each chapter. The book is by all means the best presentation of plant biology that has come to the attention of the reviewer. Used in conjunction with a corresponding book on elementary animal biology, as is the author's intention, it would make a very strong course. The zoölogical portion of the course at Cambridge covers the matter of heredity and evolution, which subjects are only briefly touched upon in this volume.—E. R. DOWNING.

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[4] TANSLEY, A. G., Elements of plant biology. London: George Allen & Unwin Ltd.; New York: Dodd, Mead & Co., pp. 410. *figs. 61*. 1922. $4.00.

NOTES FOR STUDENTS

Geotropism.—During the last few years a number of papers dealing with this phenomenon have appeared. CHOLODNYJ[5] has advanced a theory of geotropism in which he connects positive and negative geotropism with the existence in the cells of negatively charged microsomes. Being large particles, they tend under the influence of gravity to become more concentrated on the under side of the cell in the case of horizontally placed organs, thus causing the accumulation there of a greater negative charge of electricity. The negative charge attracts the metallic ions that are present, and they move to the lower part of each cell. Since the univalent ions migrate more rapidly than the bivalent, however, the ratio of univalent ions to bivalent ions changes, becoming smaller in the upper part of the cell, and larger in the under part. Reasoning from facts already established as to the colloidal and physiological effects of univalent and bivalent ions, the author draws a picture showing how this change in the ratio causes the two sides of the cell to differ in certain physical properties, and how permeability differences are set up, so that the under side of the cell grows faster than the upper. Thus negative geotropism results. In the case of positive geotropism, it is assumed that the microsomes are of different sizes, and that there is a sorting of them under the influence of gravity. This sets up a stronger electromotive force in the upper part of the cell. The theory is frankly advanced as a working hypothesis, and is evidently intended to stimulate research.

JACCARD[6] asks the question whether the unilateral growth of the trunks of trees, which results when a tree is displaced from its normal position with regard to gravity and which brings it back into its proper alignment with gravity, is the result of geotropic or phototropic stimulus perceived by the apex, and transmitted back to the growing region, or whether the stimulus results from the tensions and compressions which are caused by the inclining of the stem. JACCARD studied a number of cases of geotropic response, and comes to the conclusion that the tensions and compressions are the main cause of unilateral growth. He recognizes, however, the complexity of the problem, and admits that the orienting forces, gravity, radiation, etc., and other forces, have a part in determining the form of plants.

LYNN[7] is able to bring about a reversal of geotropic response of *Helianthus* seedlings, by subjecting them to various percentages of carbon dioxide, and offers this in support of SMALL'S[8] hydrion theory of geotropism.

[5] CHOLODNYJ, N., Zur Theorie des Geotropismus. Beih. Bot. Centralb. **39**:222–230. 1922.

[6] JACCARD, M. P., Sur le mecanisme du redressement geotropique de la tige des arbres. Rev. Gen. Bot. **34**:433–441, 481–488, 529–537. 1922.

[7] LYNN, M. J., The reversal of geotropic response in the stem. I. The effects of various percentages of carbon dioxide. New Phytol. **20**:116–122. 1921.

[8] SMALL, J., A theory of geotropism with some experiments on the chemical reversal of geotropic response in stem and root. New Phytol. **19**:49–63. 1920.

Attempts to repeat this work with *Helianthus* have not been successful in producing reversal of normal geotropism, however, and the results reported by LYNN should be confirmed before placing reliance upon them. SCHULZ,[9] in the case of flowers, finds that the peduncles perceive and react geotropically. Removing the bud or any of its important parts, or wounding the bud destroys the normal action in response to gravity. Experiments with other plants are also reported. TRONDLE[10] shows that in the case of *Lepidium* radicles, the sine law holds without any limitation. DARWIN doubted whether fern fronds had the power to respond to gravity, but PRANKERD[11] shows that ferns have this power, and that the reason that DARWIN did not detect it was owing to their unusually long reaction time, and to the fact that they early lose their geotropic irritability.

This brief reference to a number of papers perhaps will show something of the nature of the present work in the field of geotropism. Our knowledge of it is still largely in the theoretical stage, and the experiments deal largely with superficial phases of the subject. With so much work going on, however, we should soon get back to some of the physical and chemical fundamentals of the phenomenon.—S. V. EATON.

Interspecific hybrids in Crepis.—A genus of many species, some of which have a low chromosome number and which may be crossed, forms promising material for an attack on some of the fundamental problems of botany: chromosome individuality, heredity, the evolution of chromosome number, and, in a limited way, the origin of species. Such a genus is *Crepis*, with species with 3, 4, 5, 6, 8, and 20 pairs of chromosomes respectively. In several of these a start has been made in the determination of specific hereditary characters. Recently, COLLINS and MANN[12] have obtained hybrids between a 3-pair and a 4-pair, and between a 4-pair and a 20-pair species.

C. capillaris has three pairs of chromosomes, one long, one short, and one intermediate. *C. setosa* has four pairs, one long with a semi-detached end, two intermediate, and one very short. In the F_1 seven chromosomes are found, the long capillaris, and the very short and the semi-detached setosas being readily distinguished.

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[9] SCHULZ, HELENE, Über Korrelationen zwischen den Blutenteilen und den geotropischen Bewegungen der Blutenschafte, nach Untersuchungen insbesondere an Papaver. Jahrb. Wiss. Bot. **60**:1–66. 1921.

[10] TRONDLE, A., Untersuchungen über das Sinusgesetz bei den geotropischen Reaktionen von Lepidium. Jahrb. Wiss. Bot. **60**:295–306. 1921.

[11] PRANKERD, T. L., On the irritability of the fronds of *Asplenium bulbiferum*, with special reference to graviperception. Proc. Roy. Soc. London B. **93**:143–152. 1922.

[12] COLLINS, J. L., and MANN, MARGARET C., Interspecific hybrids in *Crepis*. II. A preliminary report on the results of hybridizing *C. setosa* Hall. with C. *capillaris* (L.) Wallr. and with *C. biennis* L. Genetics **8**:212–232. *figs. 9.* 1923.

In somatic characters, *C. setosa* is predominant in nearly all respects. In the pollen mother cells the chromosomes are unpaired in diakinesis, and the first meiotic division is very irregular. Three, 4, 5, or 6 microspores may result, and no back crosses were obtained when the F_1 was used as the pollen parent. Some of the ovules are functional, however, and five plants were obtained on crossing back with *C. setosa*. Two had 10, two 8, and one 7 chromosomes in root tip cells. Only one of these has flowered, and here the reduction division occurs normally. Indeed the plant is typically *C. setosa* in general and in chromosome appearance. The plant with 7 chromosomes resembles the F_1 in every way. Although the authors make no mention of it, one would suspect apogamy here. Some interesting speculations are made as to the possibilities with the other forms. The results obtained are in general accord with similar work on such hybrids.

The *setosa*×*biennis* cross revealed some distinctly new phenomena. The disparity in chromosome number, 4–20, is surprising. Stranger still is it that the reduction division should be found so nearly normal. It has been considered that in hybrids between species of different chromosome numbers the maternal and paternal chromosomes always pair, leaving the extra chromosomes as univalents, but here as many as 10 bivalents are readily distinguished in diakinesis. It is evident, therefore, that since *C. setosa* contributes but four, those from *C. biennis* must have paired with one another. This indicates a qualitative similarity among what would be considered non-homologous chromosomes, and suggests that *C. biennis* is a polyploid species. The authors assume that it is octoploid, 5 being the basic number.

The univalents lag in the anaphase, and are irregularly distributed. F_2 plants have 24 or 25 chromosomes. It may be possible to produce stable 11-pair or 12-pair species. Back crosses with each parent have been obtained, from which it is hoped to obtain stable races with such haploid chromosome numbers as 10, 12, 14, 16, and 30. It would thus appear that breaks in chromosome number series may be caused by hybridization rather than by non-disjunction.

This report is remarkable as much for its speculations, which are in a fair way to be tested, as for its actual data. Further information will be awaited with interest.—R. O. Earl.

Aluminum and iron in relation to root rots.—Hoffer and Carr[13] have attempted to establish a connection between the root rots of corn and the presence of accumulations of aluminum and iron in the nodal tissues of the plants. Characteristic purplish brown discolorations of the vascular bundles at the nodes are not always associated with fungous infection, but can be

[13] Hoffer, G. N., and Carr, R. H. Jr., Accumulation of aluminum and iron compounds in corn plants, and its probable relation to root rots. Jour. Agric. Res. **10**:801–823. 1923.

produced artificially by injection of aluminum and iron salts into healthy plants, and are believed to be due to the accumulated metallic compounds.

Soils lacking in calcium and available phosphates are especially likely to produce root rotted corn, and the diseased stalks are found richer in iron and aluminum than the healthy stalks. Treatment of soils with acid phosphate helps to overcome the tendency to root rot, and potash is particularly valuable in preventing nodal injuries.

Various soils have been tested by HOFFER and TROST,[14] who report that the roots of plants grown in soils treated with aluminum chloride were very badly rotted by *Fusarium moniliforme*. They find that different strains of corn vary in their ability to absorb aluminum, and that strains which absorb the metals most freely are more susceptible to inoculations of root rot organisms.

The evidences for accumulation of iron in the nodes are drawn from qualitative colorometric tests, which are not very convincing. These observations should have been supported by macrochemical analyses which would have proved or disproved the presence of greater amounts of iron in the nodes of plants showing discolorations. Unpublished results of experiments conducted in the Hull Botanical Laboratory show that stalks infected with *Diplodia zeae* as the root rotting organism, always take up less aluminum than healthy plants.—J. C. IRELAND.

Nitrate and top-root growth ratios.—The effect of increased nitrate content of the nutrient solution upon the ratio of top growth to root growth has been investigated by TURNER,[15] who finds that in barley and corn the ratio of tops to roots is increased significantly with the increase in nitrate concentration of the nutrient solution. The effect is independent of total concentration, and hydrogen ion concentration of the solution. Flax, however, failed to show such an effect of nitrates. The nitrates do not retard root growth directly, but will increase the root growth if carbohydrates are present for the root nutrition with the nitrates. The effects noted are explained by supposing that the increased nitrate supply leads to greater use of carbohydrates by the tops, and this deprives the roots of part of their necessary carbohydrates. A relative reduction of root growth follows.—R. B. DUSTMAN.

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[14] HOFFER, G. N., and TROST, J. F., Accumulation of aluminum and iron compounds in corn plants, and its probable relation to root rots. II. Jour. Amer. Soc. Agron. **15**:323–331. 1923.

[15] TURNER, J. W., Studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to root growth in seed plants. Amer. Jour. Bot. **9**:415–445. 1922.